(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
4 August 2005 (04.08.2005)

PCT

(10) International Publication Number
WO 2005/071073 A1

(51) International Patent Classification[7]: C12N 9/48, C07K 14/705, A61K 38/48, A61P 37/06, 37/08

(21) International Application Number: PCT/US2005/000709

(22) International Filing Date: 10 January 2005 (10.01.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/535,577 9 January 2004 (09.01.2004) UA

(71) Applicant *(for all designated States except US)*: **POINT THERAPEUTICS, INC.** [US/US]; 125 Summer Street, Suite 1840, Boston, MA 02111 (US).

(72) Inventors; and

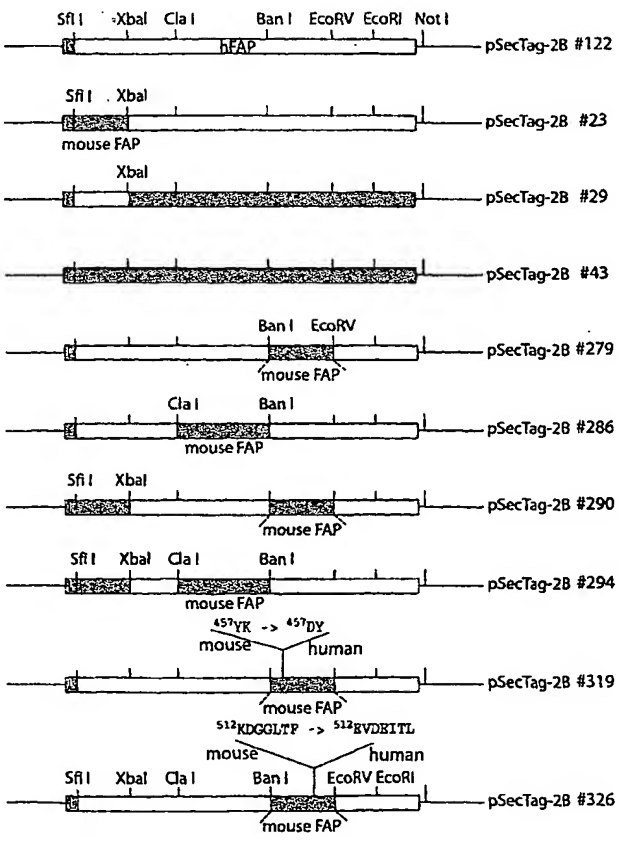
(75) Inventors/Applicants *(for US only)*: **MCLEAN, Paul, A.** [US/US]; 8 Amory Street, #3, Cambridge, MA 02139 (US). **JONES, Barry** [US/US]; 80 Wendell Street, #3, Cambridge, MA 02138 (US). **MILLER, Glenn, T.** [US/US]; 63 Bearhill Road, Merrimac, MA 01860 (US). **JESSON, Michael, I.** [US/US]; 19 Plain Street, Hopedale, MA 01747 (US).

(74) Agent: **TREVISAN, Maria, A.**; Wolf, Greenfield & Sacks, P.C., 600 Atlantic Avenue, Boston, MA 02210 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

[Continued on next page]

(54) Title: FAP COMPOSITIONS AND THE USE THEREOF FOR IMMUNOMODULATION



(57) Abstract: The invention provides compositions and methods for down-regulating immune responses using FAP alpha dimer enzyme.

WO 2005/071073 A1

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

FAP COMPOSITIONS AND THE USE THEREOF FOR IMMUNOMODULATION

Field of the Invention

The invention relates to compositions of fibroblast activation protein (FAP) alpha dimer enzyme for use in immunomodulation, and more particularly immunoinhibition.

Background of the Invention

Dipeptidyl peptidase activity is characterized by the cleavage of dipeptides from the ends of polypeptides. CD26/dipeptidyl peptidase IV (DPPIV) and fibroblast activation protein alpha (FAP alpha) are integral membrane proteins present on the cell surface of certain mammalian cell types.[28-31] Both proteins are enzymes that cleave amino-terminal dipeptides where the penultimate amino acid is either proline or alanine. CD26/DPPIV has been shown to digest biologically active polypeptides such as chemokines and short polypeptide hormones in experimental systems.[32] The biologically relevant targets of FAP have hitherto not been identified.

There exist structural similarities between the catalytic sites of CD26/DPP-1V and FAP as well as overlapping substrate specificities. However, notwithstanding the shared specificity for polypeptides with amino-terminal dipeptides of the sequence X-Pro (Ala), where X can be any natural L-amino acid, FAP was initially distinguished from CD26/DPPIV by its ability to completely digest gelatin[35]. The pattern of tissue expression of FAP also differs from CD26/DPPIV. FAP alpha is selectively expressed in reactive stromal fibroblasts of epithelial cancers, granulation tissue of healing wounds, and malignant cells of bone and soft tissue sarcomas. Normal adult tissues are generally FAP alpha-negative, but some fetal mesenchymal tissues transiently express the molecule. FAP demonstrates a restricted normal tissue distribution and abundant expression in the stroma of over 90% of breast, colorectal, and lung carcinomas. In contrast, CD26/DPPIV expression is widespread in healthy tissues. For example, it can readily be detected in epithelial and lymphoid tissue. In the latter, thymus-derived lymphocytes (T cells) have been shown to express the protein in greater amounts when the cells become activated by antigenic stimulation[28,37]. Whether FAP or CD26 is involved in regulating a particular biologically active polypeptide by N-terminal degradation might be determined by difference in anatomical expression or subtle differences

in substrate specificity between the two enzymes: such as preference for certain N-terminal residues preceding Pro/Ala[35], polypeptide size[38] or post-translational modification[39].

Summary of the Invention

The invention relates in part to methods for increasing FAP alpha dimer enzyme activity and compositions of FAP alpha dimer enzyme. In accordance with the invention, increases in FAP alpha dimer enzyme activity levels have therapeutic benefit, for example, in immunomodulation, and more particularly immunoinhibition. Some methods provided herein harness the natural ability of FAP alpha dimer enzyme to modulate IL-1 production in vivo. The invention further provides compositions of FAP alpha dimer enzyme that can be used, inter alia, in the methods of the invention.

Thus, in one aspect, the invention provides a method for down-regulating an immune response comprising administering to a subject in need thereof a FAP alpha dimer enzyme in an amount effective to down-regulate an immune response.

In one embodiment, the immune response is an IL-1 mediated condition. In another embodiment, the immune response is an abnormal immune response such as but not limited to inflammation, autoimmune disease, sepsis, graft versus host disease, transplant rejection, toxic shock syndrome, allergy, asthma, atherosclerosis, osteoarthritis, and Guillain-Barre's syndrome. In another embodiment, the abnormal immune response is subsequent to an infection, such as but not limited to an RSV infection. The autoimmune disease may be selected from the group consisting of rheumatoid arthritis, insulin dependent diabetes (type I diabetes), inflammatory bowel disease, autoimmune thyroiditis, systemic lupus erythematosus (SLE), uveitis, hemolytic anemias, rheumatic fever, Crohn's disease, Guillain-Barre's syndrome, psoriasis, Graves' disease, myasthenia gravis, glomerulonephritis, autoimmune hepatitis and multiple sclerosis.

In one embodiment, the subject does not have cancer and/or the subject does not have a predisposition to cancer.

In one embodiment, the method further comprises administering to the subject a second agent. The second agent may be an anti-inflammatory agent, an immunosuppressant, or an anti-infective agent, but it is not so limited. The anti-infective agent may be an anti-bacterial agent, an anti-viral agent, an anti-fungal agent, an anti-parasitic agent, or an anti-mycobacterial agent.

In another embodiment, the FAP alpha dimer enzyme is wild type FAP alpha dimer enzyme. The FAP alpha dimer enzyme may be a truncation mutant or a fusion or chimeric protein. The fusion or chimeric protein may comprise a sequence selected from the group consisting of a secretion sequence, a purification sequence, an epitope, a linker, a protein degradation sequence, a protease cleavage site, a self-cleaving affinity tag, a tissue localization sequence and a peptide or protein ligand. Examples of secretion sequences include but are not limited to a G-CSF leader sequence or an Ig-kappa leader sequence. Examples of purification sequences include but are not limited to GST sequence tag, a hexahistidine or polyhistidine tag, a Protein A tag, a biotin tag, a chitin tag, and a maltose binding domain. Examples of epitopes include but are not limited to a hemaglutinnin tag, a FLAG tag, a V5 tag, a myc tag and a T7 tag. The protein degradation sequence may be a PEST sequence but it is not so limited. Examples of protease cleavage site include but are not limited to enterokinase, factor Xa protease, thrombin, TEV protease, PreScission protease, Furin, and Genenase.

Unless otherwise indicated, the point mutations recited herein correspond to the amino acid of human FAP, as indicated in SEQ ID NO: 2.

In one embodiment, the fusion or chimeric protein comprises an amino acid substitution of Q732E or N733D.

In another embodiment, the FAP alpha dimer enzyme is a heterodimer. The heterodimer may be a heterodimer of a FAP alpha monomer and a DPPIV/CD26 monomer, but it is not so limited.

In another embodiment, the FAP alpha dimer enzyme comprises an amino acid substitution (as compared to or relative to wild type FAP alpha dimer amino acid sequence). The amino acid substitution may be present in the β-propeller domain. The amino acid substitution may be at positions Y124, A207, A347, G349, F351 or V352. Specific examples of amino acid substitutions include but are not limited to Y124H, A207S, A347V, G349R, F351R and V352P.

In another embodiment, the amino acid substitution is present in the catalytic domain. As an example, the amino acid substitution may be in amino acid A657, such as A657D. The amino acid substitution may be Y124H or A207S. Other examples of amino acid substitutions include but are not limited to A347V, G349R, F351R or V352P.

In still another embodiment, the amino acid substitution is present in the entrance to the catalytic site. In a related embodiment, the entrance to the catalytic site is an apical entrance. The amino acid substitution may be selected from the group consisting of G64D, Q65H, V299A, D301Q, T354E, V356H, S358T, Y359L, F401E, R402A, V403L, Q405S, T452S, A453V, D457K and Y458E. In another embodiment, the entrance is a side entrance, and optionally the amino acid substitution may be selected from the group consisting of N49K, G50N, F52Y, S53R, Y54L, T56L, F57Y, F58S, P59L, S71Q, D73E, S91E, R93S, M95F, K96D, S97E, V98F, N99G, A100H, S116Y, D117N, S119V, L121Q and Y124H.

In yet another embodiment, the amino acid substitution is present at an N-linked glycosylation site. Examples of N-linked glycosylation site is selected from the group consisting of N49, N92, N99, N227, N314 and N679. The amino acid substitution may also be at T51, T94, S101, T229, S316 or T681. In particular embodiments, the amino acid substitution is at N227 and T229. In still another embodiment, the amino acid substitution is T229M.

In any of the foregoing embodiments, the FAP alpha dimer enzyme may also be soluble and have the recited mutations overlayed thereon.

In another embodiment, the amino acid substitution alters disulfide bond formation. For example, the amino acid substitution may introduce a disulfide bond. In another embodiment, the amino acid substitution is selected from the group consisting of H378C and A386C. The amino acid substitution may be selected from the group consisting of L48C, N742C, M683C and I713C. In another related embodiment, the amino acid substitution removes a disulfide bond.

In one embodiment, the FAP alpha dimer enzyme is PEGylated. In a related embodiment, the FAP alpha dimer enzyme is PEGylated at a lysine or at a cysteine. For example, the FAP alpha dimer enzyme may be PEGylated at a cysteine introduced at position 95, 161, 173, 191, 219, 334, 372, 382, 436, 437, 445, 460, 486, 492, 499, 505, 509, 510, 521, 532, 533 564, 583, 591, 606, 616, 642, 670, 678, 715, 753, 91, 148, 263, 323, 343, or 444 (relative to wild type sequence). In another embodiment, the PEGylated FAP alpha dimer enzyme comprises a mutation in one or more amino acid positions selected from a group consisting of 95, 161, 173, 191, 219, 334, 372, 382, 436, 437, 445, 460, 486, 492, 499, 505, 509, 510, 521, 532, 533 564, 583, 591, 606, 616, 642, 670, 678, 715, 753, 91, 148, 263, 323, 343 and 444.

In another embodiment, the FAP alpha dimer enzyme is a dimerization domain mutant. In a related embodiment, the dimerization domain mutant lacks residues comprised of P232-I250 of wild type FAP alpha dimer enzyme and comprises residues P234-V254 of wild type DPPIV. In another embodiment, the dimerization domain mutant lacks residues F706-D731 of wild type FAP alpha dimer enzyme or some portion thereof and comprises residues F713-D738 of wild type DPPIV or some portion thereof. In still another embodiment, the dimerization domain mutant comprises an amino acid substitution of T248C. In another embodiment, the FAP alpha dimer enzyme lacks residues N679-N733 from wild type FAP alpha dimer enzyme and comprises residues N685-D739 of wild type DPPIV.

In yet another embodiment, the amino acid substitution is present in the cytoplasmic domain. The FAP alpha dimer enzyme may lack a cytoplasmic domain.

In still a further embodiment, the amino acid substitution is present in the transmembrane domain. The FAP alpha dimer enzyme may lack a transmembrane domain. The FAP alpha dimer enzyme may lack a cytoplasmic and transmembrane domain. For example, the FAP alpha dimer enzyme lacks residues corresponding to 1-37 from wild type FAP alpha dimer enzyme.

In preferred embodiments, the FAP alpha dimer enzyme is soluble.

In some embodiments, the FAP alpha dimer enzyme comprises an amino acid substitution of T229M. In other embodiments, it does not.

In some embodiments, the FAP alpha dimer enzyme comprises an amino acid sequence of SEQ ID NO: 4 or SEQ ID NO: 61, which may optionally be overlayed with one or more of the mutations discussed herein.

The FAP alpha dimer enzyme may be administered as a protein or as a nucleic acid.

In another embodiment, IL-1 is IL-1 alpha or IL-1 beta.

In another aspect, the invention provides a pharmaceutical preparation comprising a FAP alpha dimer enzyme in a pharmaceutically acceptable carrier, wherein the preparation is sterile and lacks an adjuvant.

In another aspect, the invention provides a pharmaceutical preparation comprising a FAP alpha dimer enzyme in a pharmaceutically acceptable carrier, and a non-adjuvant second agent.

In one embodiment, the non-adjuvant second agent is an anti-inflammatory agent or an immunosuppressant. In another embodiment, the preparation is sterile.

In various embodiments, the FAP alpha dimer enzyme is wild type FAP alpha dimer enzyme. The FAP alpha dimer enzyme may also be a truncation mutant. In yet another embodiment, the FAP alpha dimer enzyme is a fusion or chimeric protein. The fusion or chimeric protein may comprise a sequence selected from the group consisting of a secretion sequence, a purification sequence, an epitope, a linker, a protein degradation sequence, a protease cleavage site, a tissue localization sequence, a peptide or protein ligand. Examples of secretion sequences include but are not limited to a G-CSF leader sequence or an Ig-kappa leader sequence. Examples of purification sequences include but are not limited to a GST sequence tag, a hexahistidine or polyhistidine tag, a Protein A tag, a biotin tag, a chitin tag, and a maltose binding domain. Examples of epitopes include but are not limited to a hemaglutinnin tag, a FLAG tag, a V5 tag, a myc tag and a T7 tag. An example of a protein degradation sequence is a PEST sequence. Examples of protease cleavage sites include but are not limited to sites recognized by enterokinase, factor Xa protease, thrombin, TEV protease, PreScission protease, Furin, Genenase. In one embodiment, the fusion or chimeric protein comprises an amino acid substitution of Q732E or N733D.

In another embodiment, wherein the FAP alpha dimer enzyme is a heterodimer. The heterodimer may be a heterodimer of a FAP alpha monomer and a DPPIV/CD26 monomer. In another embodiment, the FAP alpha dimer enzyme comprises an amino acid substitution (as compared to or relative to wild type FAP alpha dimer).

In one embodiment, the amino acid substitution is present in the β-propeller domain. In a related embodiment, the substitution is at Y124, A207, A347, G349, F351, V352, and can include but is not limited to Y124H, A207S, A347V, G349R, F351R, V352P.

In another embodiment, the amino acid substitution is present in the catalytic domain. In a related embodiment, the amino acid substitution is selected from the group consisting of Y124H, A207S, A347V, G349R, F351R, V352P and A657D.

In still another embodiment, the amino acid substitution is at A657. In a related embodiment, the amino acid substitution is A657D. The amino acid substitution may be Y124H or A207S. The amino acid substitution may also be A347V, G349R, F351R or V352P.

In another embodiment, the amino acid substitution is present in the entrance to the catalytic domain. The entrance to the catalytic domain may be an apical entrance. The amino acid substitution may be selected from the group consisting of G64D, Q65H, V299A, D301Q,

T354E, V356H, S358T, Y359L, F401E, R402A, V403L, Q405S, T452S, A453V, D457K and Y458E. The entrance to the catalytic domain may be a side entrance. The amino acid substitution may be selected from the group consisting of N49K, G50N, F52Y, S53R, Y54L, T56L, F57Y, F58S, P59L, S71Q, D73E, S91E, R93S, M95F, K96D, S97E, V98F, N99G, A100H, S116Y, D117N, S119V, L121Q and Y124H.

In another embodiment, the amino acid substitution may be present at an N-linked glycosylation site. The N-linked glycosylation site may be selected from the group consisting of N49, N92, N99, N227, N314 and N679.

In one embodiment, the amino acid substitution is T229M.

In some preferred embodiments, the FAP alpha dimer enzyme is soluble.

In still another embodiment, the amino acid substitution alters disulfide bond formation. For example, the amino acid substitution may introduce a disulfide bond. In a related embodiment, the amino acid substitution is selected from the group consisting of H378C and A386C. In another related embodiment, the amino acid substitution is selected from the group consisting of L48C, N742C, M683C and I713C. As another example, the amino acid substitution removes a disulfide bond.

In another embodiment, FAP alpha dimer enzyme is PEGylated.

In yet another embodiment, FAP alpha dimer enzyme is a dimerization domain mutant. In a related embodiment, the dimerization domain mutant lacks residues P232-I250 of wild type FAP alpha dimer enzyme and comprises residues P234-V254 of wild type DPPIV. In another embodiment, the dimerization domain mutant lacks residues F706-D731 of wild type FAP alpha dimer enzyme and comprises residues F713-D738 of wild type DPPIV. In yet another embodiment, dimerization domain mutant comprises an amino acid substitution of T248C. In yet another embodiment, the FAP alpha dimer enzyme lacks residues N679-N733 from wild type FAP alpha dimer enzyme and comprises residues N685-D739 of wild type DPPIV.

In still another embodiment, the amino acid substitution is present in the cytoplasmic domain. The amino acid substitution may be present in the transmembrane domain. In one embodiment, the FAP alpha dimer enzyme lacks a cytoplasmic domain and/or a transmembrane domain. In a related embodiment, the FAP alpha dimer enzyme lacks residues corresponding to 1-37 from wild type FAP alpha dimer enzyme (SEQ ID NO: 70).

The FAP alpha dimer enzyme may comprise an amino acid substitution of T229M.

In various embodiments, the FAP alpha dimer enzyme comprises an amino acid sequence of SEQ ID NO: 4, SEQ ID NO: 61 or SEQ ID NO:70, optionally overlayed with one or more of the amino acid substitutions or other mutations recited herein.

In still another embodiment, the FAP alpha dimer enzyme is present in an amount effective to down-regulate an immune response.

In still another aspect, the invention provides a composition comprising a FAP alpha dimer enzyme comprising an amino acid sequence of SEQ ID NO: 61 or SEQ ID NO: 70, and optionally (1) one or more amino acid substitutions selected from the group consisting of Y124H, A207S, A347V, G349R, F351R, V352P, A657D, Q732E, N733D, G64D, Q65H, V299A, D301Q, T354E, V356H, S358T, Y359L, F401E, R402A, V403L, Q405S, T452S, A453V, D457K, Y458E, N49K, G50N, F52Y, S53R, Y54L, T56L, F57Y, F58S, P59L, S71Q, D73E, S91E, R93S, M95F, K96D, S97E, V98F, N99G, A100H, S116Y, D117N, S119V, L121Q, Y124H, H378C, A386C, L48C, N742C, M683C, I713C and T248C, (2) lacking residues P232-I250 and comprising residues P234-V254 of wild type DPPIV, (3) lacking residues F706-D731 and comprising residues F713-D738 of wild type DPPIV, (4) lacking residues N679-N733 and comprising residues N685-D739 of wild type DPPIV, or (5) an amino acid substitution of T229M.

In one embodiment, the first 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 N-terminal amino acids in SEQ ID NO: 61 are deleted.

In one embodiment, the FAP alpha dimer enzyme is a fusion or chimeric protein. The fusion or chimeric protein may comprise a sequence selected from the group consisting of a secretion sequence, a purification sequence, an epitope, a linker, a protein degradation sequence, a protease cleavage site, a self-cleaving affinity tag, a tissue localization sequence and a peptide or protein ligand. The secretion sequence may be a G-CSF leader sequence or an Ig-kappa leader sequence. The purification sequence may be selected from the group consisting of a GST sequence tag, a hexahistidine or polyhistidine tag, a Protein A tag, a biotin tag, a chitin tag, and a maltose binding domain. The epitope may be selected from the group consisting of a hemaglutinnin tag, a FLAG tag, a V5 tag, a myc tag and a T7 tag. The protein degradation sequence may be a PEST sequence. The protease cleavage site may be selected from the group consisting of enterokinase, factor Xa protease, thrombin, TEV protease, PreScission protease, Furin, and Genenase.

In one embodiment, the FAP alpha dimer enzyme is a heterodimer. The heterodimer may be a heterodimer of a FAP alpha monomer and a DPPIV/CD26 monomer.

In another embodiment, the amino acid substitution is A657D.

In yet another embodiment, the amino acid substitution is Y124H or A207S.

In still another embodiment, the amino acid substitution is A347V, G349R, F351R or V352P.

In yet a further embodiment, the amino acid substitution is selected from the group consisting of G64D, Q65H, V299A, D301Q, T354E, V356H, S358T, Y359L, F401E, R402A, V403L, Q405S, T452S, A453V, D457K and Y458E or from the group consisting of N49K, G50N, F52Y, S53R, Y54L, T56L, F57Y, F58S, P59L, S71Q, D73E, S91E, R93S, M95F, K96D, S97E, V98F, N99G, A100H, S116Y, D117N, S119V, L121Q and Y124H.

In important embodiments, the FAP alpha dimer enzyme is soluble.

The FAP alpha dimer enzyme may lack residues P232-I250 and may comprise residues P234-V254 of wild type DPPIV.

In one embodiment, the dimerization domain mutant lacks residues F706-D731 and comprises residues F713-D738 of wild type DPPIV.

In another embodiment, the FAP alpha dimer enzyme lacks residues N679-N733 and comprises residues N685-D739 of wild type DPPIV.

In yet another aspect, the invention provides a composition comprising a FAP alpha dimer enzyme comprising an amino acid substitution of A657D. In one embodiment, the FAP alpha dimer enzyme is soluble. In another embodiment, the FAP alpha dimer enzyme further comprises an amino acid substitution of T229M. In yet another embodiment, the FAP alpha dimer enzyme further comprises an amino acid substitution of Y124H or A207S. In still another embodiment, the FAP alpha dimer enzyme further comprises an amino acid substitution of A347V, G349R, F351R or V352P.

In one embodiment, the FAP alpha dimer enzyme is a fusion or chimeric protein. Various embodiments of fusion or chimeric proteins have been recited above and apply to this aspect of the invention.

In another embodiment, the heterodimer is a heterodimer of a FAP alpha monomer and a DPPIV/CD26 monomer.

In yet another embodiment, the amino acid substitution is A347V, G349R, F351R or V352P.

The FAP alpha dimer enzyme may further comprise an amino acid substitution of G64D, Q65H, V299A, D301Q, T354E, V356H, S358T, Y359L, F401E, R402A, V403L, Q405S, T452S, A453V, D457K or Y458E or an amino acid substitution of N49K, G50N, F52Y, S53R, Y54L, T56L, F57Y, F58S, P59L, S71Q, D73E, S91E, R93S, M95F, K96D, S97E, V98F, N99G, A100H, S116Y, D117N, S119V, L121Q or Y124H.

In one embodiment, the FAP alpha dimer enzyme lacks residues P232-I250 and comprises residues P234-V254 of wild type DPPIV. In another embodiment, the dimerization domain mutant lacks residues F706-D731 and comprises residues F713-D738 of wild type DPPIV. In still another embodiment, the FAP alpha dimer enzyme lacks residues N679-N733 and comprises residues N685-D739 of wild type DPPIV.

In a further aspect, the invention provides a composition comprising a FAP alpha dimer enzyme lacking amino acids 269-448 and comprising amino acids 269-448 from mouse FAP.

These and other objects of the invention will be described in further detail in connection with the detailed description of the invention.

Brief Description of the Sequence Listing

SEQ ID NO: 1 is the nucleotide sequence of human wild type FAP alpha dimer (GenBank Accession Number NM_004460).

SEQ ID NO: 2 is the amino acid sequence of human wild type FAP alpha dimer (GenBank Accession Number NM_004460).

SEQ ID NO: 3 is the nucleotide sequence of a human soluble FAP alpha dimer enzyme as contained in plasmid #122.

SEQ ID NO: 4 is the amino acid sequence of a human soluble FAP alpha dimer enzyme as coded in plasmid #122 (having vector derived DAAQPA at the N-terminus, a "TKRA" at the FAP derived N-terminal sequence due to the primer used, and a T229M mutation).

SEQ ID NO: 5 is the nucleotide sequence of wild type murine FAP alpha dimer enzyme (GenBank Accession Number Y10007).

SEQ ID NO: 6 is the amino acid sequence of wild type murine FAP alpha dimer enzyme (GenBank Accession Number Y10007).

SEQ ID NO: 7 is the amino acid sequence of hDPPIV dimerization region 1 loop.

SEQ ID NO: 8 is the amino acid sequence of hFAP alpha dimer enzyme dimerization region 1 loop.

SEQ ID NO: 9 is the amino acid sequence of hDPPIV dimerization region 2 loop.

SEQ ID NO: 10 the amino acid sequence of hFAP alpha dimer enzyme dimerization region 2 loop.

SEQ ID NO: 11 the nucleotide sequence of PCR primer hFAP1 (CCACGCTCTG AAGACAGAAT TAGC).

SEQ ID NO: 12 is the nucleotide sequence of PCR primer hFAP2 (TCAGATTCTG ATAGAGGCTTGC).

SEQ ID NO: 13 is the nucleotide sequence of PCR primer Sfi-FAP-B (GTAGTCGGCC CAGCCGGCCA CAAAGAGAGC TCTTACCCTG AAGGATATTT TAAATG).

SEQ ID NO: 14 is the amino acid sequence of the N-terminal six amino acids in mature soluble FAP alpha dimer enzyme derived from the vector pSecTag2-B (DAAQPA).

SEQ ID NO: 15 is the amino acid sequence of the N-terminal residues in FAP alpha dimer enzyme derived from the vector pSecTag2-B (i.e., excludes 6 vector-derived amino acids shown as SEQ ID NO: 14, and having a "TKRA" at the FAP derived N-terminus due to the primer used) (TKRALTLKDILNG).

SEQ ID NO: 16 is the amino acid sequence of the first 51 amino acids of wild-type hDPPIV N-terminus excerpted from GenBank Accession NM_010074: (MKTPWKVLLG LLGAAALVTI ITVPVVLLNK GTDDATADSR KTYTLTDYLKN).

SEQ ID NO: 17 is the amino acid sequence of serum DPPIV N-terminal sequence #1 (SRKTYTLTDYLKN).

SEQ ID NO: 18 is the serum DPPIV N-terminal sequence #2 (RKTYTLTDYLKN).

SEQ ID NO: 19 is the first 50 amino acids of wild-type hFAP N-terminus excerpted from GenBank Accession NM_004460 (SEQ ID NO: 2) (MKTWVKIVFG VATSAVLALL VMCIVLRPSR VHNSEEN TMRALTLKDILNG)

SEQ ID NO: 20 is the amino acid sequence of the proposed soluble hFAP alpha dimer enzyme N-terminus without the 6 amino acids imparted by the vector (TMRALTLKDILNG).

SEQ ID NO: 21 is amino acid sequence of the first 50 amino acids of wild-type mouse FAP alpha dimer enzyme N-terminus excerpted from GenBank Accession Y10007 (excerpted from SEQ ID NO: 6) (MKTWLKTVFG VTTLAALALV VICIVLRPSR VYKPEGN TKRALTLKDILNG).

SEQ ID NO: 22 is the amino acid sequence of the proposed soluble mFAP alpha dimer enzyme N-terminus without the 6 amino acids imparted by the vector (TKRALTLKDILNG).

SEQ ID NO: 23 is the nucleotide sequence upstream of the SfiI site of pSecTag2 vector (InVitrogen) showing the published signal cleavage site is between the 11th and 12th codons as follows:

(GTA CTG CTG CTC TGG GTT GCA GGT TCC ACT GGT GAC GCG GCC CAG CCG GCC).

SEQ ID NO: 24 is the amino acid sequence in the region of the signal cleavage site and SfiI site in the vector pSecTag2 (VLLLWVPGSTGDAAQPA).

SEQ ID NO: 25 is the amino acid sequence encoded by Sfi-FAP-B primer and having the murine "TKRA" at the FAP N-terminus (g DAAQPATKRA LTLKDILNG).

SEQ ID NO: 26 is nucleotide sequence of the PCR primer hG-CSF F primer (CCAAGCTG GCTAGC CACCATG GCTGGAC CTGCCACCCAGAG).

SEQ ID NO: 27 is nucleotide sequence of the hG-CSF leader-R primer (GGC TTC CTG CAC TGT CCA GAG TGC ACT).

SEQ ID NO: 28 is nucleotide sequence of the hG-CSF_FAP-F primer (GCACTCTGGA CAGTGCAGGA AGCC ACAAAG AGAGCTCTTA CCcTGAAGGA TATTTTA).

SEQ ID NO: 29 is the nucleotide sequence of the XbaI site such as hFAP-ClaI-R (GCA GGG TAA GTG GTA TCG ATA ATA AAT ATC CG).

SEQ ID NO: 30 is the nucleotide sequence of the PCR primer Sfi-DPPIV (GTAGTCGGCC CAGCCGGCC AGTCGCAAAA CTTACACTCT AACTGATTAC TTAAAAAAT).

SEQ ID NO: 31 is the nucleotide sequence of the PCR primer DPP4-R (GTCGGAGCGG CCGCCTAAGG TAAAGAGAAA CATTGTTTTA TGAAGTG).

SEQ ID NO: 32 is the nucleotide sequence of the PCR primer A657D Forward mutagenic internal (TCCAGCTGGG AATATTACGA CTCTGTCTAC ACAGAGAGAT T).

SEQ ID NO: 33 is the nucleotide sequence of the PCR primer Reverse A657D mutagenic internal (AAT CTC TCT GTG TAG ACA GAG TCG TAA TAT TCC CAG CTG GA).

SEQ ID NO: 34 is the nucleotide sequence of the PCR primer hFAP-RV-F (forward) (TAG ATG GAA ATT ACT TAT GGT ACA AGA TGA TTC TTC C).

SEQ ID NO: 35 is the nucleotide sequence of the PCR primer hFAP-Not-R (reverse) (GGT CGC TCA GCG GCC GCT TAGTC TGA CAA AGA GAA ACA CTG CTT TAG).

SEQ ID NO: 36 is the nucleotide sequence of the PCR primer Y124H-F (TTTGTATATC TAGAAAGTGA TTATTCAAAG CTTTGGAGAC ACTCTTACACA G).

SEQ ID NO: 37 is the nucleotide sequence of the PCR primer A207S-R (CCA GAG AGC ATA TTT TGT AGA AAG CAT TTC CTC TTC).

SEQ ID NO: 38 is the nucleotide sequence of the PCR primer A207S-F (GAAGAGGAAATGCTTTCTACAAAATATGCTCTCTGG).

SEQ ID NO: 39 is the nucleotide sequence of the PCR primer hFAP-Cla-F (CGG ATA TTT ATT ATC GAT ACC ACT TAC CCT GC).

SEQ ID NO: 40 is the nucleotide sequence of the PCR primer R356-R. primer (TGA AGG CCT AAA TCT TCC AAC CCA TCC AGT TCT GCT TTC TTC TAT ATGCTCC)

SEQ ID NO: 41 is the nucleotide sequence of the PCR primer R356-F (TGGGTTGGAA GATTTAGGCC TTCAACACC AGTTTTCAG CTATGATG)

SEQ ID NO: 42 is the nucleotide sequence of the PCR primer hFAP-RV-R (CTGTATTTGCTGTTAAT TGG ATA TCTTACCTTGCAAGCACAGAAAACATT).

SEQ ID NO: 43 is the nucleotide sequence of the PCR primer hFAP-RV-F (TAGATGGAAA TTACTTATGG TACAAGATGA TTCTTCC).

SEQ ID NO: 44 is the nucleotide sequence of the PCR primer DEDH-R (AATGTGGTAC TCTGACGAAG ACCACGGCTT ATCCGGCCTG T).

SEQ ID NO: 45 is the nucleotide sequence of the PCR primer DEDH-F (TGGTCTTCGT CAGAGTACCA CATTGCCTGG).

SEQ ID NO: 46 is the nucleotide sequence of the PCR primer pSecTag-R (GGCGCTATTC AGATCCTCTT CTGAGAT).

SEQ ID NO: 47 is the nucleotide sequence of the PCR primer FAP-DPP4-RI-F (GGATGATAAT CTTGAGCAC TATAAGAATT CAACAGTCAT GAGCAGAGCT).

SEQ ID NO: 48 is the nucleotide sequence of the PCR primer DPP-FAP-R (AGG CCG GAT AAG CCA TGG TCT TCA TCA GTA TAC CAC ATT GCC TGG A).

SEQ ID NO: 49 is the nucleotide sequence of the PCR primer DPP-FAP-F (CAATGTGGTA TACTGATGAA GACCATGGCT TATCCGGCCT GTCCAC).

SEQ ID NO: 50 is the nucleotide sequence of the PCR primer DPP4-A663-F (TCC CGG TGG GAG TAC TAT GCC TCA GTG TAC ACA GA).

SEQ ID NO: 51 is the nucleotide sequence of the PCR primer DPP4-A663-R (TCT GTG TAC ACT GAG GCA TAG TAC TCC CAC CGG GA).

SEQ ID NO: 52 is the nucleotide sequence of the PCR primer DPPIV 1300-F (AAGACTGCAC ATTTATTACA AAAGGCACC).

SEQ ID NO: 53 is the nucleotide sequence of the PCR primer SwaI-F (GACATTTATG ATTTAAATAA AAGGCAGCTG ATTAC AGAA GAG).

SEQ ID NO: 54 is the nucleotide sequence of the PCR primer R356-R (CTG AAG CGA AAA AAC CTC CAG CCC AGC CAG TAG TAC TCA TTC AAT G).

SEQ ID NO: 55 is the nucleotide sequence of the PCR primer R356-F (GCTGGAGGTT TTTTCGCTTC AGAACCTCAT TTTACCCTTG ATGGT).

SEQ ID NO: 56 is the nucleotide sequence of the PCR primer DPPIV_BspEI-R sequencing primer (TAG TAC TGA CAC CTT TCC GGA TTC AGC TCA).

SEQ ID NO: 57 is the nucleotide sequence of the PCR primer H124Y-R (GCCTTTTATTTAAAT CAT AAA TGT CAT ATG AAG CTG TGT AGG AAT aCC TCC ATT).

SEQ ID NO: 58 is the nucleotide sequence of the PCR primer S209A-R (ACC ACC ACA GAG CAG CGT AGG CAC TGA AGA CT).

SEQ ID NO: 59 is the nucleotide sequence of the PCR primer S209A-F (AGTCTTCAGT GCCTACTATG CTCTGTGGTG GT).

SEQ ID NO: 60 is the nucleotide sequence of the PCR primer mFAP45 (TTC CAT TGG GCC CAC GTG GTG).

SEQ ID NO: 61 is the amino acid sequence of a human soluble FAP alpha dimer enzyme (corresponding to SEQ ID NO:2 minus N-terminal amino acids 1-26).

SEQ ID NO: 62 is the consensus amino acid sequence from the alignment of hFAP dimerization region 1 and hDPP4 dimerization region 1.

SEQ ID NO: 63 is the consensus amino acid sequence from the alignment of hFAP dimerization region 2 and hDPP4 dimerization region 2.

SEQ ID NO:64 is the DNA sequence corresponding to the hFAP sequence shown in Figure 1.

SEQ ID NO:65 is the nucleotide sequence of the Sfi-FAP-B primer that encodes SEQ ID NO:25.

SEQ ID NO:66 is the amino acid sequence corresponding to the hDPPIV sequence shown in Figure 2.

SEQ ID NO:67 is the nucleotide sequence corresponding to the hDPPIV sequence of GenBank accession number NM_001935.

SEQ ID NO:68 is the nucleotide sequence corresponding to the murine DPPIV sequence of GenBank accession number NM_010074.

SEQ ID NO:69 is the amino acid sequence corresponding to the murine DPPIV sequence of GenBank accession number NM_010074.

SEQ ID NO: 70 is the amino acid sequence of a human soluble FAP alpha dimer enzyme (corresponding to SEQ ID NO:2 minus N-terminal amino acids 1-37).

Brief Description of the Drawings

FIG. 1 illustrates the amino acid and corresponding coding nucleotide sequences for each monomer that contributes to wild type human FAP alpha dimer enzyme.

FIG. 2 is an alignment of the amino acid sequences of each monomer that contributes to wild type human FAP alpha dimer enzyme and human DPPIV.

FIG. 3A is a bar graph showing the requirement of IL-1 signaling for chemokine and cytokine responses to PT-100.

FIG. 3B is a bar graph showing the requirement of IL-1 signaling for chemokine and cytokine responses to PT-100.

FIG. 4A is a graph showing G-CSF response to PT-100 in mice is undiminished in the absence of CD26 in vivo.

FIG. 4B is a graph showing TARC response to PT-100 in mice is undiminished in the absence of CD26 in vivo.

FIG. 4C is a graph showing KC response to PT-100 in mice is undiminished in the absence of CD26 in vivo.

FIG. 4D is a graph showing MIP-1 beta response to PT-100 in mice is undiminished in the absence of CD26 in vivo.

FIG. 4E is a graph showing eotaxin response to PT-100 in mice is undiminished in the absence of CD26 in vivo.

FIG. 5 is a bar graph showing the level of soluble FAP alpha dimer enzyme produced from transfected 293T cells.

FIG. 6 is a bar graph showing the ability of F19 antibody to recognize and bind to soluble human, but not mouse, FAP alpha dimer enzyme from culture supernatants of transfected 293T cells.

FIG. 7 is a graph showing inhibition of soluble human FAP alpha dimer enzyme by PT-100.

FIG. 8 is a graph comparing enzyme inhibition of soluble human FAP alpha dimer enzyme and native human FAP alpha using PT-100.

FIG. 9 shows of soluble secreted FAP alpha dimer enzyme activity in tissue culture supernatant from plasmids #23, #29 and #43 measured by production of fluorescence from Ala-Pro-AFC substrate at pH 8.1.

FIG. 10 shows FAP alpha dimer enzyme and DPPIV activity in several harvests of tissue culture supernatant from plasmids #122 and #135 respectively.

FIG. 11 shows results of inhibition of FAP alpha dimer enzyme from plasmid #217, 219, 251, 255, 257, 233 and 245 by Val-nitriloPro compared to FAP alpha dimer enzyme (#122) and DPPIV (#135).

FIG. 12A shows the pH activity profile of FAP alpha dimer enzyme comprising the A657D amino acid substitution (plasmid #233).

FIG. 12B shows the IC50 of FAP alpha dimer enzyme comprising the A657D amino acid substitution (plasmid #233) for val-boroPro.

FIG. 12C shows the binding kinetics of Val-boroPro to the FAP alpha dimer enzyme comprising the A657D amino acid substitution (plasmid #233).

FIG. 12D shows the binding kinetics of Val-boroPro to the FAP alpha dimer enzyme encoding in plasmid #122.

FIG. 12E shows the activity versus Ala-Pro-AFC substrate concentration for Km determination of human FAP alpha dimer enzyme from plasmid 122.

FIG. 12F shows the activity versus Ala-Pro-AFC substrate concentration for Km determination of human FAP alpha dimer enzyme comprising the A657D amino acid substitution (plasmid 233).

FIG. 13A shows the pH activity profile of DPPIV mutant comprising the D663A amino acid substitution (plasmid #266).

FIG. 13B shows the IC50 of DPPIV mutant comprising the D663A amino acid substitution (plasmid #266) for Val-boroPro with simultaneous addition of substrate and inhibitor.

FIG. 13C shows the binding kinetics of Val-boroPro to the DPPIV mutant comprising the D663A amino acid substitution (plasmid #266).

FIG. 13D shows the binding kinetics of Val-boroPro to wild type human DPPIV (plasmid #135).

FIG. 13E shows the activity versus Ala-Pro-AFC substrate concentration for Km determination of the DPPIV mutant comprising the D663A amino acid substitution (plasmid #266).

FIG. 14 shows the IC50 determination of hFAP alpha dimer enzyme with Q732E and N733D amino acid substitutions (plasmid #94) compared to wild-type FAP alpha dimer enzyme (plasmid #122) for Val-boroPro and Val-nitriloPro inhibitors.

FIG. 15 shows Eadie-Hoftsee plots for determination of Km for FAP-DPPIV chimera produced from plasmid #155 in tissue culture supernatant compared to control FAP alpha dimer enzyme (#122). The gradient is the negative value of the Km.

FIG. 16A is a series of maps of plasmids encoding representative human-mouse chimeras of soluble FAP alpha dimer enzyme.

FIG. 16B is a bar graph showing the relative activity of tissue culture supernatants of representative human-mouse chimeras of soluble FAP alpha dimer enzyme.

FIG. 16C is a bar graph showing the relative activity of tissue culture supernatants of representative human-mouse chimeras of soluble FAP alpha dimer enzyme.

It is to be understood that the Figures are not required for enablement of the invention.

Detailed Description of the Invention

The invention provides compositions such as pharmaceutical preparations comprising FAP alpha dimer enzyme, as well as methods of using such compositions in order to modulate immune responses.

The invention is premised, in part, on the observation that treatment of bone marrow derived stromal cells in vitro with the boronic dipeptide, Val-boroPro[40] (PT-100), has been shown to increase the levels of IL-1 beta in tissue culture supernatants after several hours of

incubation (see Example 1). Both FAP and CD26 can be detected in bone marrow derived stromal cell isolated in tissue culture. However, the effect of PT-100 appears to result from the inhibition of FAP and not CD26 because IL-1 beta levels increased in response to PT-100 in cultures of bone marrow stromal cell derived from Fischer D^- rats bearing a mutation of the *CD26* gene[41] (see Example 1) and also in spleens of mice with a knockout in the CD26 gene (see Example 1.2).

Accordingly, FAP alpha dimer enzyme appears to restrain the production of IL-1 because the production of IL-1 has been found to be increased in vitro and in vivo when the enzymatic activity of FAP alpha dimer enzyme is inhibited, as described herein. FAP alpha dimer enzyme possesses dipeptidyl peptidase activity, and inhibition of this activity with PT-100, either in a culture system containing bone marrow derived stromal cells or in vivo in mice, caused significantly increased IL-1 production in numerous experiments. If IL-1 production can be increased by blockade of FAP alpha dimer enzyme, it follows that IL-1 production should be reduced by an increase in the level of FAP alpha dimer enzyme. As will be discussed in greater detail below, FAP alpha dimer enzyme encompasses wild-type and mutant FAP alpha dimer enzymes, membrane bound as well as soluble FAP alpha dimer enzymes, heterodimers comprising FAP and a second, related molecule such as DPPIV, and the like.

Thus, as will be discussed below, the invention contemplates in a general sense methods for down-modulating an immune response. In most instances, the immune response is an abnormal immune response, an example of which is hyperimmunity. In some instances, immune response down-modulation can result in the treatment or amelioration of a particular condition associated with the immune response. Down-modulation is effected by increasing the level of FAP alpha dimer enzyme (and as a result FAP alpha dimer enzymatic activity) in a subject.

FAP alpha dimer enzyme:

The invention contemplates the use of FAP alpha dimer enzyme. As used herein, "FAP alpha dimer enzyme" refers to a protein having FAP alpha dimer activity. Wild type FAP alpha dimer has been reported to possess a number of activities including dipeptidyl peptidase activity, collagenase/gelatinase activity, and extracellular matrix degradation activity. Any of these activities may be used to screen putative FAP alpha dimer enzymes for

use in the methods of the invention. An example of a dipeptidyl peptidase assay for tracking FAP alpha dimer enzymatic activity is provided in the Examples. Collagenase/gelatinase activity and extracellular matrix degradation activity can be assayed as described by Aoyama A et al. 1990. Proc Natl Acad Sci USA. 87:8296-300; Monsky WL et al. 1994. Cancer Res. 54:5702-10. As used herein "FAP alpha dimer enzyme activity" refers to at least the dipeptidyl peptidase activity of wild type FAP alpha dimer enzyme. All mutations described herein (particularly with respect to FAP alpha dimer enzyme amino acid sequence) are relative to human wild type amino acid sequence provided as SEQ ID NO: 2. In addition, the aligned human and mouse wild type FAP amino acid sequences have the same numbering (as used herein) up to residue 736 out of a total of 760 (human) or 761 (mouse) amino acids.

Recombinant FAP has reportedly been produced by two methods in the prior art. Firstly, full-length cell membrane bound recombinant FAP has been expressed in mammalian cell lines[35] and in insect cells with an additional N-terminal His-tag (Sun et al. 2002, Protein Expr. Purif. 24, 274-281). Full-length membrane-bound enzyme has several disadvantages in that detergents are needed for its extraction from the cell membrane. Detergents present in the solubilized material are undesirable at least because they are not typically pharmaceutically acceptable. In addition, removal of detergent generally triggers undesirable agglutination or the formation of higher order oligomers due to the presence of the hydrophobic membrane-spanning domain. In contrast, soluble FAP alpha dimer enzyme avoids these limitations.

Secondly, a soluble recombinant chimeric CD8-mouse FAP protein form has been reported consisting of mouse CD8 residues 1-189 and FAP residues 27-761 (ref. 35). The CD8 portion forms a disulfide bond, which serves to keep the FAP dimerized. The first 26 amino acids of FAP including the transmembrane domain were removed. Whereas this form is soluble and avoids the use of detergents, it has the disadvantage that each monomer molecule is significantly larger by an extra 162 amino acids relative to wild type FAP alpha dimer enzyme, and that properties of the added sequence, for example binding to CD8 ligands, may perturb activity or localization of the soluble enzyme. Also, expression of the fusion in an insect cell line may have altered the glycosylation residues in an uncontrolled fashion, which is significant since the activity of FAP alpha dimer enzyme towards some substrates, notably gelatin, appear to be influenced by glycosylation. The compositions provided by the invention demonstrate that the CD8-mediated dimerization is dispensable

since stable soluble FAP alpha dimer enzyme can be made without addition of extraneous means of dimerization as described in the Examples.

Wild type:

As used herein, wild type FAP alpha dimer enzyme refers to full length FAP alpha dimer enzyme which is comprised of two wild type monomeric units. Full-length cDNA for FAP alpha monomer has been cloned previously. The human wild type FAP alpha monomer has an amino acid sequence of SEQ ID NO: 2 as derived from GenBank Accession Number NM_004460. The amino acid sequence (and corresponding coding nucleotide sequence) of the monomeric units that contribute to wild type human FAP alpha dimer enzyme are shown in FIG. 1. Wild type FAP alpha dimer enzyme is an integral cell surface membrane protein having a cytoplasmic domain (amino acids 1 to 6 in SEQ ID NO: 2), a transmembrane domain (amino acids 7 to 26 in SEQ ID NO: 2), and an extracellular domain (amino acids 27 to 760 in SEQ ID NO: 2). These various regions as well as other structural features of wild type FAP alpha dimer enzyme are illustrated in FIG. 2, which provides an alignment of amino acid sequences of FAP alpha and DPPIV monomers. Wild type FAP alpha monomer is approximately 48% identical to wild type CD26 monomer at the amino acid level. FAP alpha and CD26 monomers form heterodimers. Full-length cell membrane bound recombinant FAP has been expressed in insect (Sun et al. 2002, Protein Expr. Purif. 24, 274-281) and mammalian cell lines[35]. However, extraction from the membrane requires the addition of detergent to solubilize the membrane and the hydrophobic membrane-spanning region of the protein[35], which leads to undesirable contaminants.

FAP does not appear to have a naturally occurring soluble counterpart, unlike CD26. The soluble form of CD26 results from apparent cleavage of the membrane-bound form. Rather FAP appears to exist as either a monomer or dimer (either with itself or with other proteins such as CD26). In its monomeric form it has a molecular weight of about 88 to about 95 kilodaltons according to SDS-PAGE, and is catalytically inactive in this form. In its dimeric form, it has a molecular weight of about 170 kilodaltons according to SDS-PAGE.

FAP alpha dimer enzyme may comprise the catalytic domain of wild type FAP alpha dimer enzyme, in whole or in part. The catalytic αβ hydrolase domain of wild type FAP alpha dimer enzyme is present at amino acids 500-760 of SEQ ID NO: 2. Activity however

appears dependent upon the presence of the β propeller domain as well, in whole or in part. The β propeller domain is present at amino acids 55-499 of SEQ ID NO: 2.

Improved forms of FAP:

FAP alpha dimer enzyme encompasses various other protein forms that possess FAP alpha dimer activity. These include but are not limited to truncated versions of the wild type protein, chimeric proteins comprising regions of other proteins grafted internally into the FAP alpha sequence, fusion proteins comprising sequence from wild type FAP alpha dimer enzyme conjugated (directly or indirectly) to sequence from one or more other proteins, fusion proteins comprising sequence from wild type FAP alpha dimer enzyme conjugated (directly or indirectly) to non-FAP alpha protein sequence (e.g., leader sequences, recombinant vector sequences, and the like), heterodimeric proteins comprising at least one monomer having FAP alpha enzymatic activity in association with another monomer, point mutants of wild type FAP alpha dimer enzyme, variants of FAP alpha dimer enzyme that comprise conservative amino acid substitutions relative to the wild type amino acid sequence, and the like.

The commonality between FAP alpha dimer enzyme species is the retention of FAP alpha dimer enzymatic activity. FAP alpha dimer enzymes can have levels of enzymatic activity that are less than (but still therapeutically useful), equal to, or greater than wild type FAP alpha dimer enzyme. In some embodiments, the enzymatic profile of FAP alpha dimer enzyme is different from wild type either in the substrate affinity, the pH sensitivity, and the like.

Examples of FAP alpha dimer enzymes include N-terminal truncations and deletions; internal point mutations, insertions and deletions; chimeras comprising FAP alpha monomers from different organisms and FAP-DPPIV chimeras; heterodimeric and monomeric forms; and inactive forms that regain activity over time. Point mutations, deletions, insertions or chimeras that alter local charge, protein solubility, stability, biological half-life, interactions with other proteins, formulation properties, shelf-life or other such property are also contemplated by the invention.

Mutations to wild type FAP alpha dimer enzyme can be prepared according to methods for altering polypeptide sequence known to one of ordinary skill in the art such as are found in references which compile such methods, e.g. *Molecular Cloning: A Laboratory*

Manual, J. Sambrook, et al., eds., Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989, or *Current Protocols in Molecular Biology*, F.M. Ausubel, et al., eds., John Wiley & Sons, Inc., New York. Additional methods are described in the Examples. For example, amino acid substitutions may be made by PCR-directed mutation, site-directed mutagenesis according to the method of Kunkel (*Proc. Nat. Acad. Sci. U.S.A.* 82: 488-492, 1985), or by chemical synthesis of a gene encoding a FAP alpha dimer enzyme. The substitutions also can be made by directly synthesizing the protein or a fragment thereof.

The activity of putative FAP alpha dimer enzymes can be tested by cloning the nucleic acid encoding the putative enzyme into a bacterial or mammalian expression vector, introducing the vector into an appropriate host cell, expressing the putative enzyme, and testing for FAP alpha dimer enzymatic activity, as described herein. Such screening strategies are described in greater detail in the Examples.

Under certain circumstances of use, it is beneficial to introduce or enhance various desirable properties into a FAP alpha dimer enzyme. These altered properties include but are not limited to singly or in combination: altered Km for substrate (i.e., lower or higher Km); differential changes in Km values for specific substrates leading to altered substrate specificity and /or selectivity; a more rapid rate of substrate turnover or rate of overall catalysis; a less restrictive pH profile or broader pH optimum; an altered (e.g., lower or higher) IC50 inhibition constant for certain inhibitors; the ability to be inactivated or essentially irreversibly inhibited by a known inhibitor; altered dimerization properties; altered gelatinase activity; altered thermal stability; altered biological half-life in serum; and the like. Another possible beneficial property is the ability to be inhibited by inhibitors containing the nitrilo group including nitrilo-analogues of proline boronic acid inhibitors in which the boronic acid moiety is replaced by a nitrilo moiety (2-nitrilo-pyrollidines), including nitrilo-analogues of X-boroPro inhibitors where X = a natural or unnatural L- or D- amino acid such as valine boroPro. These nitrilo inhibitors are capable of inhibiting DPPIV (Ashworth, D.M. et al. Biorganic and Medicinal Chemistry Letters. 2-cyanopyrrolidines as potent, stable inhibitors of dipeptidyl peptidase IV. 1996. vol. 6, 1163-1166), but by comparison are less effective against wild-type FAP (vide infra).

Truncated and soluble forms:

FAP alpha dimer enzymes are preferably soluble in nature. One approach to generating a soluble FAP alpha dimer enzyme is to truncate the extracellular domain. Accordingly, some species of FAP alpha dimer enzyme corresponds to truncated forms of the wild type protein. Preferably, these forms are truncated at the N-terminus and include truncation of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39 or 40 amino acids. For example, SEQ ID NO: 61 lacks amino acids 1-26 of hFAP. Deletion of the first 37 amino acids in SEQ ID NO: 2, in whole (e.g., SEQ ID NO: 70 , which lacks amino acids 1-37) or in part, can be made without significant loss to activity. The Examples elaborate on such FAP alpha dimer enzyme forms. These truncated forms may include the cytoplasmic domain and the extracellular domain fused to each other, and thus would lack the transmembrane domain. Alternatively, they may lack both the cytoplasmic and transmembrane domain. These latter forms, as well as other forms described herein, may further comprise additional amino acids that are not derived from wild type FAP alpha dimer enzyme. Truncations at the N terminus are preferred.

Soluble forms of FAP alpha dimer enzyme are also contemplated by the invention. As used herein, a soluble FAP alpha dimer enzyme is a FAP alpha dimer enzyme that is not cell membrane associated. Soluble FAP alpha dimer enzyme can be made by removing part or all of the transmembrane domain (as described above), and optionally fusing a secretory signal sequence to it to effect secretion of the protein outside the cell. The Examples describe the generation of a soluble FAP alpha dimer enzyme which lacks the wild type FAP alpha dimer enzyme N-terminal sequence which is involved in anchoring the protein to the cell membrane. In one such embodiment, the N-terminus of the resulting FAP alpha dimer enzyme therefore starts at any residue between amino acid 25-38 inclusive of wild type human FAP alpha dimer enzyme based on numbering in SEQ ID NO: 2 that starts at the proposed methionine start codon. The soluble FAP alpha dimer enzyme retains enzymatic activity, as shown in the Examples.

In another embodiment, a soluble version of FAP alpha dimer enzyme is made in which the transmembrane domain is wholly or partially deleted, or some of its residues mutated to more hydrophilic ones thereby preserving its native N-terminus, but abrogating the membrane localization of the protein and rendering it soluble.

Fusions:

A FAP alpha dimer enzyme can be a fusion protein of FAP alpha dimer enzyme sequence conjugated to non-FAP alpha amino acid sequence. "Non-FAP alpha amino acid" refers to amino acid sequence that does not exist in wild type FAP alpha dimer enzyme. It may however exist in other known proteins (i.e., it may not be a random sequence). Non-FAP alpha amino acid sequences may comprise one or more amino acid residues. Examples of non-FAP alpha amino acid sequences include amino acid sequences of a non-FAP alpha protein domain (in whole or in part), a signal or leader secretion sequence (e.g., a G-CSF leader sequence), a purification domain or sequence (e.g., a GST sequence tag, a hexahistidine or polyhistidine tag, a Protein A tag, a biotin tag, a chitin tag, or a maltose binding domain), an epitope (e.g., a hemaglutinnin tag, a FLAG tag, a V5 tag, a myc tag, or a T7 tag), a linker, a protein degradation sequence (e.g., a PEST sequence), a protease cleavage site, a self-cleaving affinity tag (e.g. Intein 1 & intein 2), a tissue localization sequence, a peptide or protein ligand that targets the protein to particular cell surface molecules, and the like.

In yet other embodiments, the non-FAP alpha tag may provide a suitable cleavage site to produce a specified N-terminal amino acid after cleavage or to facilitate removal of the non-FAP portions (e.g. a purification domain or sequence or an epitope tag) after for example purification. Examples of proteases suitable for cleavage of recombinant FAP dimer include enterokinase, factor Xa protease, thrombin, TEV protease, PreScission protease, Furin, Genenase as described by LaVallie et al. (1994). In *Current Protocols in Molecular Biology.* pp. 16.4.5-16.4.17, John Wiley and Sons, Inc, New York, NY; Stevens *Structure*, 8: R177-R185 (2000); Cameron, A. et al. (2000) *J. Biol. Chem.* 275, 36741–36749; Krysan DJ et al. J Biol Chem. 1999 274, 23229-34; and Carter, P. et al. (1989) *Proteins: Structure, Function, and Genetics* 6, 240-248.

Examples of target molecules for peptide ligands include integrins, intercellular adhesins, addressins, various GPI-linked molecules, C-type and other lectins, cytokine and chemokine receptors and the like. Protein or epitope sequence may also be attached at either the N- or C-terminus to aid purification, localization or detection of the FAP alpha dimer enzyme. As described above, the various tags may also be attached via a sequence that provides a proteolytic site for removal of said tag after purification. In other embodiments, a

tag is attached at the N- or C-terminus to enhance therapeutic efficacy of the FAP alpha dimer enzyme.

Non-FAP alpha amino acid sequences may be fused upstream of the first FAP-derived residue or to the C-terminal of the protein. The resultant protein may be expressed either cytoplasmically or secreted, with secretion made possible by fusion to a suitable secretion sequence. The fusion point between FAP and secretion sequences preferably allows for cleavage of the secretion sequences to give the desired FAP-derived N-terminal amino acid in the mature secreted form. Secretion sequences typically are derived from type I transmembrane proteins or secreted proteins such as immunoglobulins, serum proteins, hormones, chemokines, cytokines, certain cytokine receptors which have single membrane spanning domains and the like.

An example of a commercially available secretion vector for mammalian expression is pSecTag2B vector (InVitrogen Corporation). The use of some vectors may add one or more N-terminal amino acids to the mature cleaved protein depending on the relative location of the cloning site and the site of leader cleavage. Thus in one embodiment, a signal sequence derived from a cytokine (e.g., G-CSF) or chemokine is fused to the FAP monomer nucleotide sequence. Examples of cytokine and chemokine genes providing leader sequences for production of secreted soluble FAP alpha dimer enzyme and DPPIV include the following: Interleukins 2, 3,4,5,6,7,8,9,10,11,12,13,15, 16, 17 and the like; cytokines such as G-CSF, GM-CSF, TGF, Tpo; chemokines from both the C-C and CXC families including MCP, MIP-1alpha, MIP-1beta, ENA-78, eotaxin, HCC-1, RANTES, TARC and also the CXXXC family exemplified by Fractaline (neurotatin). Sequences for these various factors can be found in publications such as "The Cytokines: Facts Book" Fitzgerald et al. Academic Press, ISBN 0-12-155142-3. Examples of cytokine receptor secretion sequences include IL-1 Type I and Type II receptors, IL-2 receptor alpha, beta or gamma chain, IL-3 alpha and IL-3 beta receptor subunits and the like, the sequences of which can be found in publications such as "The Cytokines: Facts Book" Fitzgerald et al. Academic Press, ISBN 0-12-155142-3.

It is understood that cytoplasmic versions of FAP alpha dimer enzymes are also embraced by the invention. In these embodiments, the cells are lysed and the soluble form of FAP alpha dimer enzyme is released and can be further isolated.

Chimeras:

As used herein with respect to FAP alpha dimer enzymes, the term "chimera" means a dimer comprised of two non-identical monomers, or a dimer comprised of at least one monomers that itself is derived from at least two different sources. The monomers could derive from different proteins, or from the same protein but different species. Examples of chimeras contemplated by the invention include FAP human-mouse chimeras made by splicing human and mouse FAP segments, and FAP-DPPIV chimeras. The latter category of chimeras can be further subdivided into (1) chimeras that are substantially DPPIV-like structurally and immunologically but with FAP-like catalytic or other enzymatic properties, (2) chimeras that are substantially FAP-like (especially enzymatically) but with some amino acid residue substitutions from DPPIV, and (3) chimeras that resemble both parent molecules in approximately equal proportions. In the case of FAP –DPPIV chimeras, the chimera may contain at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% FAP residues. In important embodiments, the chimera comprises the catalytic domain of wild type FAP alpha dimer enzyme either in whole or in part. It alternatively comprises substitutions, additions or deletions in the catalytic domain that do not abrogate FAP alpha dimer enzymatic activity.

Another preferred embodiment is FAP alpha dimer enzyme or a protein that is substantially wild type FAP alpha dimer enzyme structurally and immunologically, but with some DPPIV-like catalytic properties including but not limited to inhibitor specificity, pH profile and other properties beneficial to the therapeutic use, or to the modulation of its therapeutic properties. Yet another preferred embodiment is DPPIV or a protein that is substantially DPPIV structurally and immunologically, but with FAP-like catalytic or other enzymatic properties.

Mutations to the catalytic domain:

FAP alpha dimer enzymes also embrace proteins having mutations in the residues lining the interior of the active site cavity. Amino acid residue changes which confer altered properties include changes in (1) active site residues involved in substrate binding or catalytic events or the internal surface of the active site cavity (e.g., amino acids L48-S63, L89-V98, N102-P107, S116-Y126, Q151-P157, Q167-L172, W199-P216, M285-T300, G345-S357, I367-G373, W395-Q405, Y410-N413, Y450-D457, Y462-Y467, I538-I558, A578-D582, L592, W621-V629, V647-V650, Y656-D657, V659-T661, Y677, D703-V705 and H733-L735) , (2) residues lining the entrance to the active site which are known to exert

electrostatic or steric influence on substrate binding or product release in enzymes described below, or (3) residues that confer structure to the active site activity or entrance such that FAP alpha dimer enzyme conformed more closely to substrates of interest or had altered biological or thermal stability. In addition, amino acid changes that affect the accessibility of water molecules would be predicted to affect solvation of substrates or products and influence reaction kinetics.

One approach to mutating wild type FAP alpha dimer enzyme is to compare its structure and sequence with a homologous enzyme such as DPPIV in an attempt to import DPPIV properties without substantially altering key desired characteristics of FAP alpha dimer enzyme. DPPIV is the closest known homolog of wild type FAP alpha dimer enzyme and it has some properties that would be desirable in FAP alpha dimer enzyme. There are over 372 amino acid differences between the two enzymes, with an even larger number of possible combinations of residues if multiple interacting amino acid residues define a particular property. Desirable DPPIV properties include thermal stability, more rapid kinetics, long serum half-life, the ability to be efficiently inhibited by inhibitors containing the nitrilo group, and the like.

In one approach, residues lining the active site cavity of DPPIV are identified by scanning the interior of the DPPIV crystal structure (atomic coordinate file PDB #1n1m from Protein Data Bank) adjacent to the bound inhibitor, using the Protein Explorer freeware. The active site cavity in DPPIV is large and the same is assumed for FAP alpha dimer enzyme. Active site cavity residues include regions of the beta propeller lining the internal channel from the apical opening, and certain residues from the C-terminal 200 amino acid portion suggested to constitute the putative catalytic domain prior to the crystal structure determination. Together, they include DPPIV amino acids L49-S64, L90-F98, I102-P109, Y118-Y128, Q153-P159, N169-V174, W201-P218, S292-T307, G352-T365, I374-G380, W402-S412, Y417-N420, Y456-K463, Y468-S473, L544-A564, G584-D588, L598, W627-V635, V653-V656, Y662-D663, V665-T667, Y683, D709-V711 and H740-A743.

By analogy, the corresponding residues of wild type FAP alpha dimer enzyme can be identified from an alignment of the two sequences. Therefore, in FAP alpha dimer enzyme, residues lining the internal cavity include L48-S63, L89-V98, N102-P107, S116-Y126, Q151-P157, Q167-L172, W199-P216, M285-T300, G345-S357, I367-G373, W395-Q405, Y410-N413, Y450-D457, Y462-Y467, I538-I558, A578-D582, L592, W621-V629, V647-V650,

Y656-D657, V659-T661, Y677, D703-V705 and H733-L735. It is reasonable to assume that these residues are located in a similar relative position in wild type human FAP alpha dimer enzyme. Differences in putative binding-site residues are candidates for targeted changes aimed at importing attributes from DPPIV into FAP alpha dimer enzyme and vice versa. Mutation of these residues in FAP alpha dimer enzyme to alter substrate recognition, hydrolysis properties, inhibition profiles or stability are important embodiments. Some of these residues are noted in FIG. 2 which shows an alignment of monomer amino acid sequences from wild type human FAP alpha dimer enzyme and wild type human DPPIV. These residues include H126, S209, V354-P359 inclusive, and D663 in DPPIV and Y124, A207, A347, G349, F351, V352 and A657 in wild type hFAP alpha dimer enzyme. The resultant changes therefore correspond to Y124H, A207S, A347V, G349R, F351R, V352P and A657D in hFAP alpha dimer enzyme and H126Y, S209A, V354A, R356G,R358F, P359V and D663A in DPPIV. FAP alpha dimer enzymes may comprise one of more of these mutations. FAP alpha dimer enzymes comprising mutation of any of the other amino acid residues contributing to the internal surface (e.g., R421, S548, R550, in particular R421G, S448K and R550D substitutions), and of the corresponding residues in DPPIV are also embodiments in the current invention. Preferably, the catalytic triad residues consisting of S624, D702 and H734 in wild type FAP alpha dimer enzyme and S630, D708 and H740 in DPPIV are not mutated. Similarly, alterations in conserved (i.e., identical) residues that retain enzymatic activity are also preferred embodiments.

By convention, amino acid substitutions are written in an abbreviated form such as for example Y124H, denoting substitution of tyrosine number 124 with histidine.

Prior research has been published on aspartate residues conserved in the presumptive catalytic domain between human and mouse DPPIV. Mutations in mouse DPPIV established that aspartate 702 is part of the catalytic triad, and that mutation of the conserved Asp 599 and 657 residues (corresponding to D605 and D663 in human) to either alanine or threonine (D599) or glycine (D657) reportedly did not affect the enzymatic properties of the mouse enzyme. As a result, it was concluded that Asp657 mutations did not significantly modify the expression or enzymatic properties of the corresponding proteins. (David, F. et al. 1993. J. Biol. Chem., 268, 17247-17252.). Therefore, based on the prior art, human DPPIV residue D663 (mouse D657) is an unlikely candidate for altering enzymatic properties. The homologous residue A657 is not however conserved in wild type human FAP dimer enzyme.

It has now been discovered according to the invention that mutation of residue A657 in FAP alpha dimer enzyme to its corresponding DPPIV amino acid (i.e., Ala to Asp) and mutation of residue D663 in DPPIV to its corresponding FAP alpha dimer enzyme amino acid (i.e., Asp to Ala) result in enzyme molecules that have new and unexpected properties, and account in large part for the observed enzymatic differences between the two enzymes.

FAP alpha dimer enzyme can also comprise mutations in residues that line the opening of the active site. Mutation of these residues can alter the kinetics of substrate binding and catalysis. There are two putative openings to the active site, based on an analysis of the crystal structure of hDPPIV (Rasmussen et al. 2003, Nature Struc. Biol. 10, 19-25; Hiramatsu et al. 2003. Acta Crystallogr. D Biol Crystallogr. 59, 595-596, and Biochem. Biophys. Res. Comm. 2003. 302, 849-854; Haffmann et al. 2003. Proc. Natl. Acad Sci USA 100, 5063-5068;Oefner et al. 2003. Acta Crystallogr. D Biol Crystallogr. 59, 1206-12; Thoma et al. 2003. Structure (Camb) 11, 947-959). One opening is located at the side of the molecule approximately near the interface of the beta-propeller domain and the catalytic domain. The other opening is located at the apex where the beta propeller loops come together. In human DPPIV, residues lining the side entrance to the active site are distributed in several regions of the primary sequence including amino acids 50-60, 72-75, 90-103, 116-127, and 740-746 in human, and 50-60, 70-73, 88-99, 111-121, and 734-740 in mouse. The corresponding human FAP alpha residues lining the side opening are approximately residues 49-59, 70-75, 90-103, 115-126, 735-741 (735-742 in mouse FAP). Examples of contemplated mutations therefore include N49K, G50N, F52Y, S53R, Y54L, T56L, F57Y, F58S, P59L, S71Q, D73E, S91E, R93S, M95F, K96D, S97E, V98F, N99G, A100H, S116Y, D117N, S119V, L121Q and Y124H. The apical opening is contributed by DPPIV residues S59-D65, S108-D110, S158-V160, S218-G220, T304-Q308, S360-D367, E408-D413, S458-A465 approximately and by inference the homologous FAP residues (dotted underline in FIG. 2). Examples of contemplated mutations include G64D, Q65H, V299A, D301Q, T354E, V356H, S358T, Y359L, F401E, R402A, V403L, Q405S, T452S, A453V, D457K and Y458E. Alterations in these and closely adjacent residues are preferred embodiments, including substituting DPPIV residues in FAP alpha dimer enzyme, as well as importing murine equivalents, e.g., GenBank Accession Number NM_010074 for wild type murine DPPIV (SEQ ID NOs: 68 and 69 for the nucleotide and amino acid sequences), GenBank Accession Number NM_001935 for wild type human DPPIV (SEQ ID NOs: 67 and 66 for the nucleotide and amino acid sequences),

and GenBank Accession Number Y10007 for wild type murine FAP alpha dimer enzyme (SEQ ID NOs: 5 and 6 for the nucleotide and amino acid sequences), into the corresponding positions of human FAP alpha dimer enzyme and human DPPIV.

Glycosylation site mutations:

FAP alpha dimer enzymes also include mutation at one or more of the six N-linked glycosylation sites in wild type human FAP alpha dimer enzyme. Four of the six potential N-linked glycosylation sites in human FAP (at amino acids 49, 92, 99, 227, 314, 679) are shared with hDPPIV (N49, N92, N314 and N679). DPPIV has 5 additional potential glycosylation sites.. Introduction or deletion of individual glycosylation sites by site-directed mutation may affect critical properties including biological-half-life, thermal stability and gelatinase activity among others. However, it has been shown according to the invention that glycosylation at N227 is not required for FAP activity. That is, the invention embraces a FAP alpha dimer enzyme comprising a T229M mutation and that therefore lacks one of the six glycosylation sites by destroying the N-x-T glycosylation motif at N227. The invention however also embraces FAP alpha dimer enzymes that are wild type at residue 229. It has been reported that wild type FAP alpha dimer enzyme expressed in COS-1 cells is differently glycosylated than in human sarcoma and fibroblasts (Scanlan et al. 1994. Proc. Natl. Acad. Sci. 91, 5657-5661), and it has further been reported that non-glycosylated FAP does not have gelatinase activity (Sun et al. 2002, Protein Expr. Purif. 24, 274-281). Thus, the FAP alpha dimer enzyme may further comprise mutations that result in the removal of one or more glycosylation sites to selectively reduce gelatinase/collagenase activity without impacting dipeptidyl peptidase activity. Compensatory additions of new glycosylation sites to preserve overall enzymatic or biological half-life, solubility and other desired properties, are also contemplated. Such compensatory additions include sites found in human DPPIV, surface lysines comprised of FAP alpha dimer enzyme residues 173, 191, 334, 372, 382, 436, 437, 445, 460, 492, 499, 505, 509, 510, 521, 532, 533 564, 583, 591, 606, 616, 642, 670, 678, 715, and 753; and K219T, or I192T which creates a N-x-T motif.

Disulfide bond mutations:

DPPIV has 5 disulfide bonds, involving cysteine residues 328/339, 385/394, 444/447, 454/472 and 649/762 denoted in pairs. DPPIV is known to be stable at temperatures up to 55°C. Wild type human FAP alpha dimer enzyme lacks equivalent Cys385 and Cys394

residues and thus has only 3 disulfide bonds (i.e., 6 of the 8 analogous cysteines of DPPIV are present). Disulfide bonds have been reported to contribute to stability in many secreted enzymes, examples of which are trypsin, chymotrypsin, lysosyme, ribonuclease and others. FAP alpha dimer enzymes may include addition of novel cysteines to potentiate disulfide bond formation in order to enhance protein stability or removal of disulfides to decrease stability depending on the therapeutic application. Examples include introduction of the cysteines equivalent to DPPIV Cys 385 and Cys394 into FAP alpha dimer enzyme by simultaneously mutating residues at or near H378 and A386 to cysteine (e.g., H378C and A386C).

In another embodiment, a disulfide bond is introduced to secure the N-terminus to one of the C-terminal residues . This is accomplished by replacement of a pair of residues that are roughly juxtaposed in the tertiary structure with cysteines. The latter then form disulfide bonds in the folded protein. One embodiment includes mutation to cysteine of one of T38 or M39 or a nearby residue and simultaneously one of N506, Q508 or H533 which tether the N-terminus of FAP alpha dimer enzyme to the C-terminal region (e.g., one of T38C, M39C, plus one of N506C, Q508C and H533C). In another embodiment, residue L48 or a nearby residue and residue N742 or a nearby residue in FAP alpha dimer enzyme are changed to cysteine for the purpose of introducing a disulfide link (e.g., L48C and N742C). Residues M683 and I713 can also be mutated to be cysteines (e.g., M683C and I713C).

In other embodiments, disulfide bonds within FAP alpha dimer enzyme are removed by eliminating one or both of the participatory cysteines to modulate the stability and thus activity and biological half-life of the protein. Preferably, pairs of cysteines are mutated thereby avoiding formation of inappropriate disulfide bonds involving the remaining cysteine residue as has been suggested in the art.

PEGylation:

FAP alpha dimer enzymes may also be PEGylated (i.e., conjugated to polyethylene glycol) in order to increase biological half-life. Modification of proteins with polyethylene glycol (PEG) can be used to reduce the immunoreactivity, prolong the clearance time (biological half-life) and improve stability of proteins. (Inada Y et al. Trends Biotechnol. 1995 13:86-91.) PEG is generally attached to proteins at a epsilon amino group of surface lysine residues, and methods have been described for altering residues to lysine in a protein to

increase the number of sites for attachment. (Hershfield MS et al. Proc Natl Acad Sci U S A. 1991. 88:7185-9.). Another method, exemplified in U.S. Patent No. 6,608,183, attaches PEG to cysteine groups that are naturally present or engineered into the protein sequence. In the embodiments contemplated here, surface residues of FAP alpha dimer enzyme including but not limited to lysine and cysteine residues are labeled with PEG. In another embodiment, surface residues which are not normally reactive to the PEG labeling reagents are altered by site directed mutagenesis to either lysine or cysteine to allow attachment of PEG.

The location of potential surface residues can be determined by analogy to the DPPIV crystal structure or empirically. In one embodiment, one or more glycosylation sites in FAP alpha dimer enzyme or DPPIV (underline in FIG. 2) are replaced by PEG by altering any one of the outside residues of the asparagine-x-serine/threonine glycosylation motifs (x= any amino acid) to lysine or cysteine for PEG attachment. In some embodiments, selected surface lysines are changed to cysteines for cysteine-PEG links. A list of examples of surface lysine candidates includes lysines at positions 95, 161, 173, 191, 219, 334, 372, 382, 436, 437, 445, 460, 486, 492, 499, 505, 509, 510, 521, 532, 533 564, 583, 591, 606, 616, 642, 670, 678, 715, and 753 in wild type FAP alpha dimer enzyme. In others, surface arginines at positions 91, 148, 263, 323, 343 and 444 in wild type FAP alpha dimer enzyme are altered to permit PEGylation.

In yet another embodiment, lysine groups are removed to prevent PEG attachment in locations not conducive to enzyme function or to reduce the number of potential attachment sites. A list of examples of surface lysine candidates includes lysines at positions 95, 161, 173, 191, 219, 334, 372, 382, 436, 437, 445, 460, 486, 492, 499, 505, 509, 510, 521, 532, 533 564, 583, 591, 606, 616, 642, 670, 678, 715, and 753 in wild type FAP alpha dimer enzyme. In the case of heterodimers or chimeras, examples of DPPIV surface lysine candidates include lysines 41, 50 , 56, 71, 139, 163, 175, 190, 250, 267, 391, 392, 399, 423, 433, 441, 463, 466, 489, 502, 512, 513, 523, 536, 538, 539, 554, 589, 615, 622, 648, 696, 721 and 760 which can be changed in any combination to arginine in one embodiment, and in another to a mixture selected from arginine, other charged amino acid (e.g., aspartate, glutamate or histidine) or polar amino acid (e.g., glycine, serine, threonine, asparagine or glutamine), but not to asparagines in cases where it would create an asparagine-x-serine/threonine glycosylation motif. In another embodiment, DPPIV lysines which line the internal cavity are changed in

any combination as follows: K71 is changed to glutamine, K463 to aspartate and K554 to serine to prevent PEG attachment. In another embodiment, DPPIV lysines 122, 258, 373 and 512; and FAP lysines 120, 254 and 366 which appear to be important structurally, are excluded from elimination. In yet another embodiment, DPPIV lysines 122, 258, 373 and 512; and FAP lysines 120, 254 and 366 are altered to arginine or histidine.

Heterodimers:

FAP alpha dimer enzymes also encompass heterodimers comprising FAP monomers and other monomers. These heterodimers may be formed by co-expression of monomers in the same cell. Heterodimer formation can be facilitated by the presence of matched dimerization domains engineered into one or both monomers as described below.

Dimerization:

FAP alpha dimer enzymes may also embrace mutation at residues involved in the obligate dimerization. In one such embodiment residues in two regions presumptively involved in dimerization, based on scanning the hDPPIV crystal structure are targeted. Region 1 corresponds approximately to hDPPIV residues P234-V254 (PLIEYSFYSDESLQYPKTVRV; SEQ ID NO: 7) which form a loop with additional extra-loop residues Y256-V262 also participating in the interface, and FAP alpha dimer enzyme residues P232-I250 (PVIAYSYYGDEQYPRTINI; SEQ ID NO: 8) which form a loop with additional extra-loop residues Y252-K258 also participating in the interface. Region 2 corresponds to DPPIV residues F713-D738 (FQQSAQISKA LVDVGVDFQA MWYTD; SEQ ID NO: 9) and FAP alpha dimer enzyme residues F706-D731 (FQNSAQIAKA LVNAQVDFQA MWYSD; SEQ ID NO: 10). Region 1 has eight and Region 2 has six amino acid differences between wild type human FAP alpha dimer enzyme and DPPIV. In addition, FAP alpha dimer enzyme has a 2 amino acid deletion in region 1 relative to DPPIV based on optimal alignment.

Region 1.

```
hFAP:   TDIPVIAYSYYGDE--QYPRTINIPYPKAGAKN 259        SEQ ID NO: 8
        T++P+I YS+Y DE  QYP+T+ +PYPKAGA N          SEQ ID NO: 62
hDPP4:  TEVPLIEYSFYSDESLQYPKTVRVPYPKAGAVN 263       SEQ ID NO: 7
```

Region 2.

```
hFAP:   692 VDYLLIHGTADDNVHFQNSAQIAKALVNAQVDFQAMWYSDQNHGL-SGLSTNHLYTHMTHFLKQCFSL 758
            V+YLLIHGTADDNVHFQ SAQI+KALV+  VDFQAMWY+D++HG+ S  +  H+YTHM+HF+KQCFSL
```

hDPPIV: 698 VEYLLIHGTADDNVHFQQSAQISKALVDVGVDFQAMWYTDEDHGIASSTAHQHIYTHMSHFIKQCFSL 765

hFAP is SEQ ID NO: 10; hDPPIV is SEQ ID NO: 9; and consensus is SEQ ID NO: 63

FAP alpha dimer enzymes can also be FAP-DPPIV chimeras in which the dimerization interfaces in the proteins, either wholly or in part, are exchanged between the proteins. For example, replacing human FAP alpha dimer enzyme dimerization region 1 with DPPIV region 1 introduces eight amino acid changes and adds a two amino acid insertion. Replacing wild type human FAP alpha dimer enzyme dimerization region 2 with wild type DPPIV region 2 introduces 6 amino acid changes. Examples include FAP-DPPIV chimeras in which, separately or together, P232-I250 of wild type FAP alpha dimer enzyme Region 1 or a portion thereof is replaced with P234-V254 of wild type DPPIV or some portion thereof; and F706-D731 of wild type FAP alpha dimer enzyme Region 2 or a portion thereof is replaced with F713-D738 of wild type DPPIV or some portion thereof. Another preferred embodiment includes analogous DPPIV-FAP chimeras in which, separately or together, some portion of P234-V254 of wild type DPPIV is replaced with P232-I250 of wild type human FAP alpha dimer enzyme and/or F713-D738 of wild type DPPIV is replaced with F706-D731 of wild type human FAP dimer enzyme, wholly or in part. Also envisioned is the introduction of cysteine residues in the dimer interface, which, depending on location, may allow inter-subunit di-sulfide bonds, or in another case, intramolecular di-sulfide bonds, either within or between dimerization Regions 1 and 2. Embodiments include mutation of residue T251 of wild type DPPIV to a cysteine (i.e., T251C) which is proximal to the T251 of the other chain of the dimer, and analogously mutation of residue T248 of wild type FAP alpha dimer enzyme to a cysteine (i.e., T248C) to induce an inter-subunit disulfide bond.

It is to be understood that the resultant chimeric monomers can then be combined in a variety of ways provided the resultant dimer possesses FAP alpha dimer enzymatic activity, as described herein.

Monomeric forms of FAP:

In another embodiment, the loops that constitute the dimerization domains (see FIG. 2) are deleted to yield monomers. Additionally, residues that form the dimer interface may be altered to reduce affinity for intermolecular interaction and thereby favor monomer over dimer forms. These latter alterations include changing hydrophobic residues to hydrophilic residues to facilitate aqueous exposure. These changes may accompany other alterations to render the protein monomeric. Other embodiments target residues 252-262 of DPPIV and

their FAP equivalents and mutation of DPPIV Y661 and FAP Y655 to hydrophilic residues such as aspartic acid, glutamic acid, asparagine, glutamine, serine, lysine, arginine, and histidine to allow solvent exposure.

Mutation of charged amino acid surface residues:

FAP alpha dimer enzymes may also comprise mutation of charged amino acid surface residues. Such mutations may change the electrostatic properties of the enzyme. For example, crystallographic studies reportedly show that the residues of wild type DPPIV facing the membrane are positively charged, presumably to complement the negative charge of membrane lipids. A similar phenomenon may apply for FAP alpha dimer enzymes. However, no such constraint need be applied to a FAP alpha dimer enzyme that is soluble (i.e., not membrane bound) and thus changes to exposed residues would be more tolerated. These exposed residues include surface lysines at positions 173, 191, 219, 334, 372, 382, 436, 437, 445, 460, 492, 499, 505, 509, 510, 521, 532, 533 564, 583, 591, 606, 616, 642, 670, 678, 715, and 753; and surface arginines at positions 91, 142, 148, 175, 263, 323, 343, 444, 530 and 691. Mutation of arginines 109, 303 and 426 is also possible.

Conservative substitutions:

The skilled artisan will also realize that FAP alpha dimer enzymes also comprise conservative amino acid substitutions relative to wild type sequence. As used herein, a "conservative amino acid substitution" refers to an amino acid substitution which does not substantially alter the relative charge in one instance, or in another retains a charge but of opposite sign, or size characteristics of the polypeptide in which the amino acid substitution is made. Exemplary conservative substitutions of amino acids include substitutions made amongst amino acids within the following groups: (a) M, I, L, V; (b) F, Y, W; (c) K, R, H; (d) A, G; (e) S, T; (f) Q, N; and (g) E, D (h) M, I, L, V, F, Y; and (i) K, R, H S, Q, N, E, D.

Inhibited forms of FAP and DPPIV which slowly recover activity:

The invention further contemplates FAP alpha dimer enzymes that are reversibly inhibited by inhibitors such as dipeptide boronic acids but with slow recovery kinetics. That is, the enzyme can regain its enzymatic activity following exposure to the inhibitor but it does so slowly as compared to wild type proteins. These FAP alpha dimer enzyme forms can be

used, inter alia, in combination with an inhibitor to control the level of enzymatic activity in a subject. For example, such forms may be provided in a pharmaceutically acceptable injectable preparation together with inhibitors, among them dipeptide proline boronic acids, at such a concentration and for such a period as necessary to allow binding of the inhibitor. Enzyme treated in this manner may be stored as necessary for intermediate periods under conditions conducive to the preservation of the inhibited complex, including freezing or cooling on ice or by other means, or by the manipulation of pH. Concentrations of approximately 0.5 nM inhibitor or higher but typically not greater than 10 μM and time periods approximately 1 -15 min or longer for complex formation are preferable but not limiting. Preferred dipeptide proline boronic acids include Val-boroPro and Ala-boroPro. The dipeptide proline boronic acids may also possess one or both amino acids in the unnatural D-configuration. Alterations to the nature of the first amino acid and the stereochemical configuration of the boronic acid to modulate the duration of inhibition and kinetics of release are contemplated embodiments. Other embodiments include mixing of inhibitors or of enzyme preparations treated with different inhibitors as a means of flattening out (i.e., plateauing) the released activity versus time profile from simple first order kinetics.

Examples of mutants suitable for slow-release formulations include FAP A657D and DPPIV D663A, among others. Also, heterodimers between different slow release mutant forms of FAP alpha dimer enzyme or with DPPIV is also envisioned.

Conditions to be treated:

The invention provides methods to increase the level of FAP alpha dimer enzymatic activity in vivo. FAP alpha dimer enzymatic activity may be increased in vivo by administering FAP alpha dimer enzymes or nucleic acids encoding such proteins.

FAP alpha dimer enzymes are used to down-regulate immune responses in vivo. In important embodiments, the immune responses are abnormal immune responses. An abnormal immune response is an immune response that is either inappropriate (e.g., is not functioning to eradicate an infection or other condition for which an immune response would be needed) or uncontrolled. An abnormal immune response in the context of the invention generally refers to hyperimmunity. Examples include inflammation and inflammatory conditions, autoimmune disease, sepsis and septic shock (e.g., endotoxic shock), cytokine induced shock, allergies or bronchitis (including chronic allergies and chronic bronchitis),

asthma, uncontrolled immune responses associated with particular infections such as RSV, graft versus host disease (GVHD), tissue, skin and organ transplantation rejection, osteoporosis, psoriasis, acute pancreatitis, premature labor secondary to intrauterine infections, chronic inflammatory pathologies with or without autoimmune involvement, fever and the like. Treatment of these conditions with FAP alpha can reduce symptoms or slow disease development.

"Inflammation" is a localised protective response elicited by a foreign (non-self) antigen, and/or by an injury or destruction of tissue(s), which serves to destroy, dilute or sequester the foreign antigen, the injurious agent, and/or the injured tissue. Inflammation generally occurs when tissues are injured by viruses, bacteria, trauma, chemicals, heat, cold or any other harmful stimuli. In such instances, T cells, B cells and macrophages work with other cells and soluble products that are mediators of inflammatory responses including neutrophils, eosinophils, basophils, kinin and coagulation systems, and complement cascade.

In another important embodiment, the inflammation is caused by an immune response against "self-antigen," and the subject in need of treatment according to the invention has an autoimmune disease. "Autoimmune disease" as used herein, results when a subject's immune system attacks its own organs or tissues, producing a clinical condition associated with the destruction of that tissue, as exemplified by diseases such as rheumatoid arthritis, uveitis, insulin-dependent diabetes mellitus, autoimmune pulmonary inflammation, hemolytic anemias, rheumatic fever, Crohn's disease, Guillain-Barre's syndrome, psoriasis, thyroiditis, Graves' disease, autoimmune thyroiditis, myasthenia gravis, glomerulonephritis, autoimmune hepatitis, multiple sclerosis, systemic lupus erythematosus, autoimmune inflammatory eye disease, etc.

Examples include chronic and acute inflammatory conditions such as but not limited to arthritis, rheumatoid arthritis, chronic inflammatory arthritis, inflammation associated with pulmonary disease, inflammatory bowel disease (e.g., ulcerative colitis and Crohn's disease), inflammation resulting from allergic reactions or acute or chronic infections (caused by viral, bacterial, fungal, protozoan or other organisms), systemic lupus erythematosus, atherosclerosis, airway inflammatory disease, tendonitis, inflammatory stage of alopecia, insect bites, multiple sclerosis, chronic inflammation in the brain and thrombotic disease, pulmonary fibrosis, psoriasis and hypersensitivity skin disease.

Arthritis is a chronic joint disease characterized by the inflammation of synovial tissue and by a progressive degradation of the molecular components constituting the joint cartilage and bone.

Inflammatory bowel disease ("IBD") refers to an acute or chronic inflammatory autoimmune condition affecting the gastrointestinal tract and associated with one or more of the following symptoms: transmural acute and chronic granulomatous inflammation with ulceration, crypt abbesses, marked fibrosis, spontaneous reactivation, extraintestinal inflammation and anemia. IBD generally refers to two distinct conditions known as ulcerative colitis and Crohn's disease. Ulcerative colitis is a mucosal ulceration of the colon. Crohn's disease, also known as ileitis, ileocolitis and colitis, is a transmural inflammation that can be found throughout the general intestinal tract.

Examples of non-human autoimmune conditions include murine experimental autoimmune encephalitis, systemic lupus erythmatosis in MRL/lpr/lpr mice or NZB hybrid mice, murine autoimmune collagen arthritis, insulin dependent diabetes mellitus in NOD mice and BB rats, and murine experimental myasthenia gravis.

The condition can also be the systemic response to diseases such as sepsis and pancreatitis. Sepsis is the systemic inflammatory response caused by microbial infection. Release of bacterial endotoxin from invading microbes stimulates the release of tumor necrosis factor alpha and IL-1, among other cytokines. Symptoms associated with sepsis include changes in thermoregulation, vascular permeability and resistance, cardiac function, bone marrow function, activity of key enzymes, drop in mean arterial blood pressure (MAP), decrease in cardiac output, tachycardia, tachypnea, lacticacidemia and leukopenia.

Subjects at risk of developing sepsis, or that have developed sepsis are treated according to the invention. One example of a subject at risk of developing sepsis is a subject that will undergo surgery.

Pancreatitis is acute or chronic inflammation of the pancreas, which may be asymptomatic or symptomatic and which is due to autodigestion of the pancreatic tissue by its own enzymes. It may be caused by alcoholism or biliary tract disease, hyperlipaemia, hyperparathyroidism, abdominal trauma, vasculitis or uraemia.

The condition can be an allergic reaction and conditions associated therewith (e.g., anaphylaxis, serum sickness, drug reactions, food allergies, insect venom allergies, mastocytosis, allergic rhinitis, hypersensitivity pneumonitis, urticaria, angioedema, eczema,

atopic dermatitis, allergic contact dermatitis, erythema multiforme, Stevens-Johnson syndrome, allergic conjunctivitis, atopic keratoconjunctivitis, venereal keratoconjunctivitis, giant papillary conjunctivitis and contact allergies), such as asthma (particularly allergic asthma) or bronchitis (including chronic bronchitis) and other respiratory problems. The allergic reaction may also be to chemical or biological substances such as penicillin.

The method of the invention may also be used to induce a state of tolerance or anergy. These latter states may be appropriate during pregnancy (to prevent a mother from developing an immune response to her child, particularly with respect to the Rh antigen), in autoimmune conditions in which the body inappropriately identifies its own organs and cells are being foreign and thus mounts an immune response against them, and in organ transplantation to prevent organ rejection. Tolerance generally refers to a state in which T cells are rendered non-responsive or anergic. It may be demonstrated by the absence of a T cell response upon subsequent exposure to a particular antigen.

IL-1 mediated conditions:

In some instances, FAP alpha dimer enzymes are administered to subjects having an IL-1 mediated condition. An IL-1 mediated condition as used herein is a disease or medical condition associated with elevated levels of IL-1 in bodily fluids or tissue.

IL-1 is a cytokine that is produced as a result of infections and various kinds antigenic stimulation. IL-1 protein has a molecular weight of about 17.5 kDa in its mature form, and is produced primarily by the macrophages but also by epidermal, lymphoid, vascular and epithelial cells. IL-1 is a key cytokine in the body's ability to mount an inflammatory and immune response. It can however also act as a hormone, inducing metabolic, neurological, hematological and endocrinological changes.

IL-1 exists in two active forms. The predominant form is IL-1 beta which is initially synthesized as an inactive precursor of 269 amino acids (31 kDa). This precursor is then cleaved to give rise to a mature form having amino acids 117-269 of the precursor form. The much less frequent form of IL-1 is IL-1 alpha which is about 26% homologous with IL-1 beta. It is initially synthesized as an active precursor form of 271 amino acids, which when cleaved gives rise to the mature form. IL-1 alpha and IL-1 beta are coded by distinct genes. IL-1 alpha and IL-1 beta recognize and bind to the same receptor on the cell surface (IL-1R).

IL-1 beta is a cytokine that acts to increase the production of other cytokines and

chemokines[1,42]. This activity is described as proinflammatory, and can contribute to the pathology of inflammatory autoimmune diseases such as rheumatoid arthritis and type I diabetes[2]. Inhibition of dipeptidyl peptidase activity in vivo by administration of PT-100 to mice induces both increased production of IL-1 beta and other cytokines and chemokines. The cytokine and chemokine responses to PT-100 require IL-1 beta signaling because, in mice which lack the IL-1 receptor, these chemokine responses were found to be greatly reduced (FIG. 3). Similarly to the in vitro response of bone marrow stromal cells, in vivo responses to PT-100 in mice appeared to be due to FAP inhibition because the induction of increased cytokine and chemokine production was undiminished in the absence of CD26 (FIG. 4). Overall, the data indicate that inhibition of FAP results in the stimulation of IL-1 beta production that in turn induces the expression of other cytokines and chemokines involved in immune and inflammatory responses.

IL-1 has been shown to play a role in many conditions. Autoimmune or inflammatory diseases in which IL-1 is involved include rheumatoid arthritis[3,4], insulin dependent diabetes (type I diabetes)[5], septic shock[6,7], inflammatory bowel disease[2], and atherosclerosis[2]. In addition, a linkage between IL-1 and disease has been suggested in transplant rejection, graft-versus-host disease (GVHD), psoriasis, asthma, osteoporosis, osteoarthritis, periodontal disease, autoimmune thyroiditis, alcoholic hepatitis, premature labor secondary to uterine infection atherosclerosis, Guillain-Barre's syndrome and sleep disorders[2,8].

Co-administration:

FAP alpha dimer enzymes can be administered to subjects either alone or in combination with other agents. As an example, FAP alpha dimer enzymes can be administered in combination with immunosuppressants, anti-inflammatory agents, anti-infectives, and the like.

In some instances, the agents are administered substantially simultaneously with each other. By "substantially simultaneously," it is meant that the FAP alpha dimer enzyme is administered to a subject close enough in time with the administration of second (preferably therapeutic) agent, whereby the second agent may exert a potentiating effect on FAP alpha dimer enzyme activity. Thus, by substantially simultaneously it is meant that the FAP alpha dimer enzyme is administered before, at the same time, and/or after the administration of the

second agent. As will be described below, FAP alpha dimer enzyme can be administered as a polypeptide, and/or a nucleic acid that encodes the polypeptide.

In certain embodiments, the second agents are immunosuppressants. An immunosuppressant is an agent that down-regulates an immune response or prevents the initiation of an immune response. These include but are not limited to Azathioprine; Azathioprine Sodium; Cyclosporine; Daltroban; Gusperimus Trihydrochloride; Sirolimus; and Tacrolimus.

In other embodiments, the second agents are anti-inflammatory agents. Anti-inflammatory agents are agents that prevent or down-regulate inflammation. These include but are not limited to Alclofenac; Alclometasone Dipropionate; Algestone Acetonide; Alpha Amylase; Amcinafal; Amcinafide; Amfenac Sodium; Amiprilose Hydrochloride; Anakinra; Anirolac; Anitrazafen; Apazone; Balsalazide Disodium; Bendazac; Benoxaprofen; Benzydamine Hydrochloride; Bromelains; Broperamole; Budesonide; Carprofen; Cicloprofen; Cintazone; Cliprofen; Clobetasol Propionate; Clobetasone Butyrate; Clopirac; Cloticasone Propionate; Cormethasone Acetate; Cortodoxone; Deflazacort; Desonide; Desoximetasone; Dexamethasone Dipropionate; Diclofenac Potassium; Diclofenac Sodium; Diflorasone Diacetate; Diflumidone Sodium; Diflunisal; Difluprednate; Diftalone; Dimethyl Sulfoxide; Drocinonide; Endrysone; Enlimomab; Enolicam Sodium; Epirizole; Etodolac; Etofenamate; Felbinac; Fenamole; Fenbufen; Fenclofenac; Fenclorac; Fendosal; Fenpipalone; Fentiazac; Flazalone; Fluazacort; Flufenamic Acid; Flumizole; Flunisolide Acetate; Flunixin; Flunixin Meglumine; Fluocortin Butyl; Fluorometholone Acetate; Fluquazone; Flurbiprofen; Fluretofen; Fluticasone Propionate; Furaprofen; Furobufen; Halcinonide; Halobetasol Propionate; Halopredone Acetate; Ibufenac; Ibuprofen; Ibuprofen Aluminum; Ibuprofen Piconol; Ilonidap; Indomethacin; Indomethacin Sodium; Indoprofen; Indoxole; Intrazole; Isoflupredone Acetate; Isoxepac; Isoxicam; Ketoprofen; Lofemizole Hydrochloride; Lornoxicam; Loteprednol Etabonate; Meclofenamate Sodium; Meclofenamic Acid; Meclorisone Dibutyrate; Mefenamic Acid; Mesalamine; Meseclazone; Methylprednisolone Suleptanate; Morniflumate; Nabumetone; Naproxen; Naproxen Sodium; Naproxol; Nimazone; Olsalazine Sodium; Orgotein; Orpanoxin; Oxaprozin; Oxyphenbutazone; Paranyline Hydrochloride; Pentosan Polysulfate Sodium; Phenbutazone Sodium Glycerate; Pirfenidone; Piroxicam; Piroxicam Cinnamate; Piroxicam Olamine; Pirprofen; Prednazate; Prifelone; Prodolic Acid; Proquazone; Proxazole; Proxazole Citrate; Rimexolone; Romazarit;

Salcolex; Salnacedin; Salsalate; Sanguinarium Chloride; Seclazone; Sermetacin; Sudoxicam; Sulindac; Suprofen; Talmetacin; Talniflumate; Talosalate; Tebufelone; Tenidap; Tenidap Sodium; Tenoxicam; Tesicam; Tesimide; Tetrydamine; Tiopinac; Tixocortol Pivalate; Tolmetin; Tolmetin Sodium; Triclonide; Triflumidate; Zidometacin; Zomepirac Sodium.

Anti-infectives include anti-bacterial agents, anti-viral agents, anti-fungal agents, anti-parasitic agents, anti-mycobacterial agents and the like.

Anti-bacterial agents kill or inhibit the growth or function of bacteria. A large class of anti-bacterial agents is antibiotics. Antibiotics, which are effective for killing or inhibiting a wide range of bacteria, are referred to as broad spectrum antibiotics. Other types of antibiotics are predominantly effective against the bacteria of the class gram-positive or gram-negative. These types of antibiotics are referred to as narrow spectrum antibiotics. Other antibiotics which are effective against a single organism or disease and not against other types of bacteria, are referred to as limited spectrum antibiotics.

Anti-bacterial agents are sometimes classified based on their primary mode of action. In general, anti-bacterial agents are cell wall synthesis inhibitors, cell membrane inhibitors, protein synthesis inhibitors, nucleic acid synthesis or functional inhibitors, and competitive inhibitors. Cell wall synthesis inhibitors inhibit a step in the process of cell wall synthesis, and in general in the synthesis of bacterial peptidoglycan. Cell wall synthesis inhibitors include β-lactam antibiotics, natural penicillins, semi-synthetic penicillins, ampicillin, clavulanic acid, cephalolsporins, and bacitracin.

The β-lactams are antibiotics containing a four-membered β-lactam ring which inhibits the last step of peptidoglycan synthesis. β-lactam antibiotics can be synthesized or natural. The natural antibiotics are generally produced by two groups of fungi, *penicillium* and *cephalosporium* molds. The β-lactam antibiotics produced by *penicillium* are the natural penicillins, such as penicillin G or penicillin V. These are produced by fermentation of *penicillium chrysogenum*. The natural penicillins have a narrow spectrum of activity and are generally effective against *streptococcus*, *gonococcus*, and *staphylococcus*. Other types of natural penicillins, which are also effective against gram-positive bacteria, include penicillins F, X, K, and O.

Semi-synthetic penicillins are generally modifications of the molecule 6-aminopenicillanic acid produced by a mold. The 6-aminopenicillanic acid can be modified by addition of side chains which produce penicillins having broader spectrums of activity than

natural penicillins or various other advantageous properties. Some types of semi-synthetic penicillins have broad spectrums against gram-positive and gram-negative bacteria, but are inactivated by penicillinase. These semi-synthetic penicillins include ampicillin, carbenicillin, oxacillin, azlocillin, mezlocillin, and piperacillin. Other types of semi-synthetic penicillins have narrower activities against gram-positive bacteria, but have developed properties such that they are not inactivated by penicillinase. These include, for instance, methicillin, dicloxacillin, and nafcillin. Some of the broad spectrum semi-synthetic penicillins can be used in combination with β-lactamase inhibitors, such as clavulamic acids and sulbactam. The β-lactamase inhibitors do not have anti-microbial action but they function to inhibit penicillinase, thus protecting the semi-synthetic penicillin from degradation.

Another type of β-lactam antibiotic is the cephalolsporins. Cephalolsporins are produced by *cephalolsporium* molds, and have a similar mode of action to penicillin. They are sensitive to degradation by bacterial β-lactamases, and thus, are not always effective alone. Cephalolsporins, however, are resistant to penicillinase. They are effective against a variety of gram-positive and gram-negative bacteria. Cephalolsporins include, but are not limited to, cephalothin, cephapirin, cephalexin, cefamandole, cefaclor, cefazolin, cefuroxine, cefoxitin, cefotaxime, cefsulodin, cefetamet, cefixime, ceftriaxone, cefoperazone, ceftazidine, and moxalactam.

Bacitracin is another class of antibiotics which inhibit cell wall synthesis. These antibiotics, produced by *bacillus* species, prevent cell wall growth by inhibiting the release of muropeptide subunits or peptidoglycan from the molecule that delivers the subunit to the outside of the membrane. Although bacitracin is effective against gram-positive bacteria, its use is limited in general to topical administration because of its high toxicity.

Carbapenems are another broad spectrum β-lactam antibiotic, which is capable of inhibiting cell wall synthesis. Examples of carbapenems include, but are not limited to, imipenems. Monobactems are also broad spectrum β-lactam antibiotics, and include, euztreonam. An antibiotic produced by *streptomyces*, vancomycin, is also effective against gram-positive bacteria by inhibiting cell membrane synthesis.

Another class of anti-bacterial agents is cell membrane inhibitors. These compounds disorganize the structure or inhibit the function of bacterial membranes. Alteration of the cytoplasmic membrane of bacteria results in leakage of cellular materials from the cell.

Compounds that inhibit or interfere with the cell membrane cause death of the cell because the integrity of the cytoplasmic and outer membranes is vital to bacteria.

One clinically useful anti-bacterial agent that is a cell membrane inhibitor is Polymyxin, produced by Bacillus polymyxis. Polymyxins interfere with membrane function by binding to membrane phospholipids. Polymyxin is effective mainly against Gram-negative bacteria and is generally used in severe *Pseudomonas* infections or *Pseudomonas* infections that are resistant to less toxic antibiotics.

Other cell membrane inhibitors include Amphotericin B and Nystatin produced by the bacterium *Streptomyces* which are also anti-fungal agents, used predominantly in the treatment of systemic fungal infections and *Candida* yeast infections respectively. Imidazoles, produced by the bacterium *Streptomyces*, are another class of antibiotic that is a cell membrane inhibitor. Imidazoles are used as anti-bacterial agents as well as anti-fungal agents, e.g., used for treatment of yeast infections, dermatophytic infections, and systemic fungal infections. Imidazoles include but are not limited to clotrimazole, miconazole, ketoconazole, itraconazole, and fluconazole.

Many anti-bacterial agents are protein synthesis inhibitors. These compounds prevent bacteria from synthesizing structural proteins and enzymes and thus cause inhibition of bacterial cell growth or function or cell death. In general these compounds interfere with the processes of transcription or translation. Anti-bacterial agents that block transcription include but are not limited to Rifampins, produced by the bacterium *Streptomyces* and Ethambutol, a synthetic chemical. Rifampins, which inhibit the enzyme RNA polymerase, have a broad spectrum activity and are effective against gram-positive and gram-negative bacteria as well as *Mycobacterium tuberculosis.* Ethambutol is effective against *Mycobacterium tuberculosis.*

Anti-bacterial agents which block translation interfere with bacterial ribosomes to prevent mRNA from being translated into proteins. In general this class of compounds includes but is not limited to tetracyclines, chloramphenicol, the macrolides (e.g. erythromycin) and the aminoglycosides (e.g. streptomycin).

Some of these compounds bind irreversibly to the 30S ribosomal subunit and cause a misreading of the mRNA, e.g., the aminoglycosides. The aminoglycosides are a class of antibiotics which are produced by the bacterium *Streptomyces,* such as, for instance streptomycin, kanamycin, tobramycin, amikacin, and gentamicin. Aminoglycosides have been used against a wide variety of bacterial infections caused by Gram-positive and Gram-

negative bacteria. Streptomycin has been used extensively as a primary drug in the treatment of *tuberculosis*. Gentamicin is used against many strains of Gram-positive and Gram-negative bacteria, including *Pseudomonas infections*, especially in combination with Tobramycin. Kanamycin is used against many Gram-positive bacteria, including penicillin-resistant *staphylococci*.

Another type of translation inhibitor anti-bacterial agent is the tetracyclines. The tetracyclines bind reversibly to the 30S ribosomal subunit and interfere with the binding of charged tRNA to the bacterial ribosome. The tetracyclines are a class of antibiotics, produced by the bacterium *Streptomyces,* that are broad-spectrum and are effective against a variety of gram-positive and gram-negative bacteria. Examples of tetracyclines include tetracycline, minocycline, doxycycline, and chlortetracycline. They are important for the treatment of many types of bacteria but are particularly important in the treatment of Lyme disease.

Anti-bacterial agents such as the macrolides bind reversibly to the 50S ribosomal subunit and inhibits elongation of the protein by peptidyl transferase or prevents the release of uncharged tRNA from the bacterial ribosome or both. The macrolides contain large lactone rings linked through glycoside bonds with amino sugars. These compounds include erythromycin, roxithromycin, clarithromycin, oleandomycin, and azithromycin. Erythromycin is active against most Gram-positive bacteria, *Neisseria, Legionella* and *Haemophilus*, but not against the *Enterobacteriaceae*. Lincomycin and clindamycin, which block peptide bond formation during protein synthesis, are used against gram-positive bacteria.

Another type of translation inhibitor is chloramphenicol. Chloramphenicol binds the 70S ribosome inhibiting the bacterial enzyme peptidyl transferase thereby preventing the growth of the polypeptide chain during protein synthesis. Chloramphenicol can be prepared from Streptomyces or produced entirely by chemical synthesis.

Some anti-bacterial agents disrupt nucleic acid synthesis or function, e.g., bind to DNA or RNA so that their messages cannot be read. These include but are not limited to quinolones and co-trimoxazole, both synthetic chemicals and rifamycins, a natural or semi-synthetic chemical. The quinolones block bacterial DNA replication by inhibiting the DNA gyrase, the enzyme needed by bacteria to produce their circular DNA. They are broad spectrum and examples include norfloxacin, ciprofloxacin, enoxacin, nalidixic acid and temafloxacin. Nalidixic acid is a bactericidal agent that binds to the DNA gyrase enzyme

(topoisomerase) which is essential for DNA replication and allows supercoils to be relaxed and reformed, inhibiting DNA gyrase activity. The main use of nalidixic acid is in treatment of lower urinary tract infections (UTI) because it is effective against several types of Gram-negative bacteria such as *E. coli*, *Enterobacter aerogenes*, *K. pneumoniae* and *Proteus* species which are common causes of UTI. Co-trimoxazole is a combination of sulfamethoxazole and trimethoprim, which blocks the bacterial synthesis of folic acid needed to make DNA nucleotides. Rifampicin is a derivative of rifamycin that is active against Gram-positive bacteria (including *Mycobacterium tuberculosis* and meningitis caused by *Neisseria meningitidis*) and some Gram-negative bacteria. Rifampicin binds to the beta subunit of the polymerase and blocks the addition of the first nucleotide which is necessary to activate the polymerase, thereby blocking mRNA synthesis.

Another class of anti-bacterial agents is compounds that function as competitive inhibitors of bacterial enzymes. The competitive inhibitors are mostly all structurally similar to a bacterial growth factor and compete for binding but do not perform the metabolic function in the cell. These compounds include sulfonamides and chemically modified forms of sulfanilamide which have even higher and broader anti-bacterial activity. The sulfonamides (e.g. gantrisin and trimethoprim) are useful for the treatment of *Streptococcus pneumoniae*, beta-hemolytic *streptococci* and *E. coli*, and have been used in the treatment of uncomplicated UTI caused by E. coli, and in the treatment of meningococcal meningitis.

Anti-viral agents are compounds which prevent infection of cells by viruses or replication of the virus within the cell. There are several stages within the process of viral infection which can be blocked or inhibited by antiviral agents. These stages include, attachment of the virus to the host cell (immunoglobulin or binding peptides), uncoating of the virus (e.g. amantadine), synthesis or translation of viral mRNA (e.g. interferon), replication of viral RNA or DNA (e.g. nucleoside analogues), maturation of new virus proteins (e.g. protease inhibitors), and budding and release of the virus.

Nucleotide analogues are synthetic compounds which are similar to nucleotides, but which have an incomplete or abnormal deoxyribose or ribose group. Once the nucleotide analogues are in the cell, they are phosphorylated, producing the triphosphate formed which competes with normal nucleotides for incorporation into the viral DNA or RNA. Once the triphosphate form of the nucleotide analogue is incorporated into the growing nucleic acid chain, it causes irreversible association with the viral polymerase and thus chain termination.

Nucleotide analogues include, but are not limited to, acyclovir (used for the treatment of herpes simplex virus and varicella-zoster virus), gancyclovir (useful for the treatment of cytomegalovirus), idoxuridine, ribavirin (useful for the treatment of respiratory syncitial virus), dideoxyinosine, dideoxycytidine, and zidovudine (azidothymidine).

Anti-fungal agents are useful for the treatment and prevention of infective fungi. Anti-fungal agents are sometimes classified by their mechanism of action. Some anti-fungal agents function as cell wall inhibitors by inhibiting glucose synthase. These include, but are not limited to, basiungin/ECB. Other anti-fungal agents function by destabilizing membrane integrity. These include, but are not limited to, imidazoles, such as clotrimazole, sertaconzole, fluconazole, itraconazole, ketoconazole, miconazole, and voriconacole, as well as FK 463, amphotericin B, BAY 38-9502, MK 991, pradimicin, UK 292, butenafine, and turbinifine. Other anti-fungal agents function by breaking down chitin (e.g. chitinase) or immunosuppression (501 cream). Still other anti-fungal agents include prednisone, disodium chromoglycat, nystatin, hydroxystilbamidine, 5-fluorocytosine, pimaricin, turbinifine, gentian violet, resorcin, iodine, thiabendazole, glutarardehyde, tolnaftate, econazole, sulfonamides, phyfluorocytozine, and oral potassium iodide.

Parasiticides are agents that kill parasites directly. Such compounds are known in the art and are generally commercially available. Examples of parasiticides useful for human administration include but are not limited to albendazole, amphotericin B, benznidazole, bithionol, chloroquine HCl, chloroquine phosphate, clindamycin, dehydroemetine, diethylcarbamazine, diloxanide furoate, eflornithine, furazolidaone, glucocorticoids, halofantrine, iodoquinol, ivermectin, mebendazole, mefloquine, meglumine antimoniate, melarsoprol, metrifonate, metronidazole, niclosamide, nifurtimox, oxamniquine, paromomycin, pentamidine isethionate, piperazine, praziquantel, primaquine phosphate, proguanil, pyrantel pamoate, pyrimethanmine-sulfonamides, pyrimethanmine-sulfadoxine, quinacrine HCl, quinine sulfate, quinidine gluconate, spiramycin, stibogluconate sodium (sodium antimony gluconate), suramin, tetracycline, doxycycline, thiabendazole, tinidazole, trimethroprim-sulfamethoxazole, and tryparsamide some of which are used alone or in combination with others.

Parasiticides used in non-human subjects include piperazine, diethylcarbamazine, thiabendazole, fenbendazole, albendazole, oxfendazole, oxibendazole, febantel, levamisole, pyrantel tartrate, pyrantel pamoate, dichlorvos, ivermectin, doramectic, milbemycin oxime,

iprinomectin, moxidectin, N-butyl chloride, toluene, hygromycin B thiacetarsemide sodium, melarsomine, praziquantel, epsiprantel, benzimidazoles such as fenbendazole, albendazole, oxfendazole, clorsulon, albendazole, amprolium; decoquinate, lasalocid, monensin sulfadimethoxine; sulfamethazine, sulfaquinoxaline, metronidazole.

Parasiticides used in horses include mebendazole, oxfendazole, febantel, pyrantel, dichlorvos, trichlorfon, ivermectin, piperazine; for *S. westeri*: ivermectin, benzimiddazoles such as thiabendazole, cambendazole, oxibendazole and fenbendazole. Useful parasiticides in dogs include milbemycin oxine, ivermectin, pyrantel pamoate and the combination of ivermectin and pyrantel. The treatment of parasites in swine can include the use of levamisole, piperazine, pyrantel, thiabendazole, dichlorvos and fenbendazole. In sheep and goats anthelmintic agents include levamisole or ivermectin. Caparsolate has shown some efficacy in the treatment of D. immitis (heartworm) in cats.

Agents used in the prevention and treatment of protozoal diseases in poultry, particularly trichomoniasis, can be administered in the feed or in the drinking water and include protozoacides such as aminonitrothiazole, dimetridazole (Emtryl), nithiazide (Hepzide) and Enheptin. However, some of these drugs are no longer available for use in agrigultural stocks in the USA.

Delivery of FAP alpha dimer enzyme:

As stated above, in some instances, soluble FAP is administered as a nucleic acid or a protein. In some embodiments, the nucleic acids or proteins/peptides are isolated. In still further embodiments, the nucleic acids or proteins/peptides are substantially pure.

As used herein with respect to nucleic acids, the term "isolated" means: (i) amplified in vitro by, for example, polymerase chain reaction (PCR); (ii) recombinantly produced by cloning; (iii) purified, as by cleavage and gel separation; or (iv) synthesized by, for example, chemical synthesis. An isolated nucleic acid is one which is readily manipulable by recombinant DNA techniques well known in the art. Thus, a nucleotide sequence contained in a vector in which 5' and 3' restriction sites are known or for which polymerase chain reaction (PCR) primer sequences have been disclosed is considered isolated but a nucleic acid sequence existing in its native state in its natural host is not. An isolated nucleic acid may be substantially purified, but need not be. For example, a nucleic acid that is isolated within a cloning or expression vector is not pure in that it may comprise only a tiny percentage of the

material in the cell in which it resides. Such a nucleic acid is isolated, however, as the term is used herein because it is readily manipulable by standard techniques known to those of ordinary skill in the art.

As used herein with respect to proteins/peptides, the term "isolated" means separated from its native environment in sufficiently pure form so that it can be manipulated or used for any one of the purposes of the invention. Thus, isolated means sufficiently pure to be used (i) to raise and/or isolate antibodies, (ii) as a reagent in an assay, or (iii) for sequencing, etc.

The term "substantially pure" means that the nucleic acid or protein/peptide is essentially free of other substances with which it may be found in nature or in vitro systems, to an extent practical and appropriate for their intended use. Substantially pure polypeptides may be produced by techniques well known in the art. As an example, because an isolated protein may be admixed with a pharmaceutically acceptable carrier in a pharmaceutical preparation, the protein may comprise only a small percentage by weight of the preparation. The protein is nonetheless isolated in that it has been separated from many of the substances with which it may be associated in living systems, i.e. isolated from certain other proteins.

The invention embraces the use of nucleic acids that encode the FAP alpha dimer enzymes described herein, including degenerates, homologs and alleles thereof.

Homologs and alleles of the FAP nucleic acids can be identified by conventional techniques. Thus, an aspect of the invention is those nucleic acid sequences which code for FAP alpha dimer enzyme and which hybridize to a nucleic acid molecule consisting of the coding region of SEQ ID NO: 1 (e.g., nucleotides 209 to 2488), under stringent conditions. The term "stringent conditions" as used herein refers to parameters with which the art is familiar. Nucleic acid hybridization parameters may be found in references which compile such methods, e.g. Molecular Cloning: A Laboratory Manual, J. Sambrook, et al., eds., Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989, or Current Protocols in Molecular Biology, F.M. Ausubel, et al., eds., John Wiley & Sons, Inc., New York. More specifically, stringent conditions, as used herein, refers, for example, to hybridization at 65°C in hybridization buffer (3.5 x SSC, 0.02% Ficoll, 0.02% polyvinyl pyrolidone, 0.02% Bovine Serum Albumin, 2.5mM NaH_2PO_4(pH7), 0.5% SDS, 2mM EDTA). SSC is 0.15M sodium chloride/0.15M sodium citrate, pH7; SDS is sodium dodecyl sulphate; and EDTA is ethylenediaminetetracetic acid. After hybridization, the membrane

upon which the DNA is transferred is washed at 2x SSC at room temperature and then at 0.1x SSC/0.1% SDS at temperatures up to 68°C.

There are other conditions, reagents, and so forth which can be used, and would result in a similar degree of stringency. The skilled artisan will be familiar with such conditions, and thus they are not given here. It will be understood, however, that the skilled artisan will be able to manipulate the conditions in a manner to permit the clear identification of homologs and alleles of FAP alpha dimer enzyme nucleic acids of the invention. The skilled artisan also is familiar with the methodology for screening cells and libraries for expression of such molecules which then are routinely isolated, followed by isolation of the pertinent nucleic acid molecule and sequencing.

In general homologs and alleles typically will share at least 75% nucleotide identity to SEQ ID NO: 3 (nucleotide sequence of the FAP portion used to make the soluble FAP and the nucleotide sequence of soluble FAP itself), and/or at least 90% amino acid identity to SEQ ID NO: 4 (amino acid sequence of the FAP portion used to make the soluble FAP and the amino acid sequence of soluble FAP itself) or SEQ ID NOs: 61 or 70. Preferably, homologs and alleles will share at least 85% nucleotide identity and/or at least 95% amino acid identity and, even more preferably, at least 95% nucleotide identity and/or at least 99% amino acid identity will be shared. The homology can be calculated using various, publicly available software tools developed by NCBI (Bethesda, Maryland) that can be obtained through the NCBI website on the internet. Exemplary software tools include the BLAST system (see NIH website) using default settings. Pairwise and ClustalW alignments (BLOSUM30 and/or BLOSUM62 matrix settings) as well as Kyte-Doolittle hydropathic analysis can be obtained using the MacVetor sequence analysis software (Oxford Molecular Group). Watson-Crick complements of the foregoing nucleic acids also are embraced by the invention.

The invention also includes degenerate nucleic acids which include alternative codons to those present in FAP alpha dimer enzyme nucleic acids provided herein. For example, serine residues are encoded by the codons TCA, AGT, TCC, TCG, TCT and AGC. Each of the six codons is equivalent for the purposes of encoding a serine residue. Thus, it will be apparent to one of ordinary skill in the art that any of the serine-encoding nucleotide triplets may be employed to direct the protein synthesis apparatus, in vitro or in vivo, to incorporate a serine residue into an elongating FAP alpha dimer enzyme. Similarly, nucleotide sequence triplets which encode other amino acid residues include, but are not limited to: CCA, CCC,

CCG and CCT (proline codons); CGA, CGC, CGG, CGT, AGA and AGG (arginine codons); ACA, ACC, ACG and ACT (threonine codons); AAC and AAT (asparagine codons); and ATA, ATC and ATT (isoleucine codons). Other amino acid residues may be encoded similarly by multiple nucleotide sequences. Thus, the invention embraces degenerate nucleic acids that differ from the biologically isolated nucleic acids in codon sequence due to the degeneracy of the genetic code.

The invention also contemplates mutations to the nucleic acids encoding FAP alpha dimer enzyme that are silent as to the amino acid sequence of the protein, but which provide preferred codons for translation in a particular host. The preferred codons for translation of a nucleic acid in, e.g., E. coli, are well known to those of ordinary skill in the art. Still other mutations can be made to the noncoding sequences of a FAP alpha dimer enzyme nucleic acid or cDNA clone to enhance expression of the polypeptide.

The methods of the invention may also utilize vectors containing the nucleic acid for FAP alpha dimer enzyme, and cells transfected with such vectors. Virtually any cells, prokaryotic or eukaryotic, which can be transformed with heterologous DNA or RNA and which can be grown or maintained in culture or which can be introduced into a subject, may be used in the practice of the invention. Examples include bacterial cells such as E. coli, insect cells, and mammalian cells such as mouse, hamster, pig, goat, primate, etc. They may be of a wide variety of tissue types, including mast cells, fibroblasts, oocytes and lymphocytes, and they may be primary cells or cell lines. Specific examples include CHO cells and COS cells. Cell-free transcription systems also may be used in lieu of cells.

As used herein, a "vector" may be any of a number of nucleic acids into which a desired sequence may be inserted by restriction and ligation for transport between different genetic environments or for expression in a host cell. Vectors are typically composed of DNA although RNA vectors are also available. Vectors include, but are not limited to, plasmids, phagemids and virus genomes. A cloning vector is one which is able to replicate in a host cell, and which is further characterized by one or more endonuclease restriction sites at which the vector may be cut in a determinable fashion and into which a desired DNA sequence may be ligated such that the new recombinant vector retains its ability to replicate in the host cell. In the case of plasmids, replication of the desired sequence may occur many times as the plasmid increases in copy number within the host bacterium or just a single time

per host before the host reproduces by mitosis. In the case of phage, replication may occur actively during a lytic phase or passively during a lysogenic phase.

An expression vector is one into which a desired DNA sequence may be inserted by restriction and ligation such that it is operably joined to regulatory sequences and may be expressed as an RNA transcript. Vectors may further contain one or more marker sequences suitable for use in the identification of cells which have or have not been transformed or transfected with the vector. Markers include, for example, genes encoding proteins which increase or decrease either resistance or sensitivity to antibiotics or other compounds, genes which encode enzymes whose activities are detectable by standard assays known in the art (e.g., beta-galactosidase or alkaline phosphatase), and genes which visibly affect the phenotype of transformed or transfected cells, hosts, colonies or plaques (e.g., green fluorescent protein). Preferred vectors are those capable of autonomous replication and expression of the structural gene products present in the DNA segments to which they are operably joined.

The FAP alpha dimer enzyme nucleic acid would commonly be placed under the control of a regulatory sequence. Regulatory sequences include, but are not limited to, promoters, and other elements which although capable of affecting transcriptional levels are not, in and of themselves, sufficient for such transcription. Examples of these latter elements include enhancers and repressor elements. Minimal promoter elements have been recognized in the art and include sequences such as a CCAAT box or a TATA sequence. Suitable marker sequences for these purposes are similar to those described above.

As used herein, a coding sequence and regulatory sequences are said to be "operably" joined when they are covalently linked in such a way as to place the expression or transcription of the coding sequence under the influence or control of the regulatory sequences. If it is desired that the coding sequences be translated into a functional protein, two DNA sequences are said to be operably joined if induction of a promoter in the 5' regulatory sequences results in the transcription of the coding sequence and if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the promoter region to direct the transcription of the coding sequences, or (3) interfere with the ability of the corresponding RNA transcript to be translated into a protein. Thus, a promoter region would be operably joined to a coding

sequence if the promoter region were capable of effecting transcription of that DNA sequence such that the resulting transcript might be translated into the desired protein or polypeptide.

The precise nature of the regulatory sequences needed for gene expression may vary between species or cell types, but shall in general include, as necessary, 5' non-transcribed and 5' non-translated sequences involved with the initiation of transcription and translation respectively, such as a TATA box, capping sequence, CCAAT sequence, and the like. Especially, such 5' non-transcribed regulatory sequences will include a promoter region which includes a promoter sequence for transcriptional control of the operably joined coding sequence. Regulatory sequences may also include enhancer sequences or upstream activator sequences as desired. The vectors of the invention may optionally include 5' leader or signal sequences. The choice and design of an appropriate vector is within the ability and discretion of one of ordinary skill in the art.

Expression vectors containing all the necessary elements for expression are commercially available and known to those skilled in the art. See, e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, 1989. Cells are genetically engineered by the introduction into the cells of heterologous nucleic acid, usually DNA, molecules, encoding a soluble FAP polypeptide or fragment or a variant thereof. The heterologous nucleic acid molecules are placed under operable control of transcriptional elements to permit the expression of the heterologous nucleic acid molecules in the host cell.

Preferred systems for mRNA expression in mammalian cells are those such as pcDNA3.1 (available from Invitrogen, Carlsbad, CA) that contain a selectable marker such as a gene that confers G418 resistance (which facilitates the selection of stably transfected cell lines) and the human cytomegalovirus (CMV) enhancer-promoter sequences. Additionally, suitable for expression in primate or canine cell lines is the pCEP4 vector (Invitrogen, Carlsbad, CA), which contains an Epstein Barr virus (EBV) origin of replication, facilitating the maintenance of plasmid as a multicopy extrachromosomal element. Another expression vector is the pEF-BOS plasmid containing the promoter of polypeptide Elongation Factor 1-alpha, which stimulates efficiently transcription in vitro. The plasmid is described by Mishizuma and Nagata (Nuc. Acids Res. 18:5322, 1990), and its use in transfection experiments is disclosed by, for example, Demoulin (Mol. Cell. Biol. 16:4710-4716, 1996). Still another preferred expression vector is an adenovirus, described by Stratford-Perricaudet,

which is defective for E1 and E3 proteins (J. Clin. Invest. 90:626-630, 1992). The use of the adenovirus as an Adeno.P1A recombinant is disclosed by Warnier et al., in intradermal injection in mice for immunization against P1A (Int. J. Cancer, 67:303-310, 1996). An example of a commercially available secretion vector for mammalian expression is pSecTag2B vector (InVitrogen Corporation). Generally, the FAP alpha dimer enzyme amino acid sequence to be expressed from these vectors should be fused to a signal sequence in order to ensure release of FAP alpha dimer enzyme.

The invention embraces the use of the above described, FAP alpha dimer enzyme nucleotide sequence containing expression vectors, to transfect host cells and cell lines, be these prokaryotic (e.g., E. coli), or eukaryotic (e.g., CHO cells, COS cells, yeast expression systems and recombinant baculovirus expression in insect cells). Especially useful are mammalian cells such as human, mouse, hamster, pig, goat, primate, etc., from a wide variety of tissue types including primary cells and established cell lines. Specific examples include mammalian epithelial cells, fibroblast cells and kidney epithelial cells, either as primary cells or cell lines. Production of recombinant FAP alpha dimer enzyme heterodimers is accomplished by transfection of the chosen cell line with a two or more plasmids which encode different forms of FAP alpha dimer enzyme, or a mixture of FAP and DPPIV expressing plasmids in proportions deemed optimal for the desired outcome.

In some instances, it may be desirable to coat or load FAP alpha dimer enzyme onto material surfaces. "Material surfaces" as used herein, include, but are not limited to, dental and orthopedic prosthetic implants, artificial valves, and organic implantable tissue such as a stent, allogeneic and/or xenogeneic tissue, organ and/or vasculature.

Implantable prosthetic devices have been used in the surgical repair or replacement of internal tissue for many years. Orthopedic implants include a wide variety of devices, each suited to fulfill particular medical needs. Examples of such devices are hip joint replacement devices, knee joint replacement devices, shoulder joint replacement devices, and pins, braces and plates used to set fractured bones. Some contemporary orthopedic and dental implants, use high performance metals such as cobalt-chrome and titanium alloy to achieve high strength. These materials are readily fabricated into the complex shapes typical of these devices using mature metal working techniques including casting and machining. In important embodiments, the material surface is part of an implant.

The material surface is coated with an amount of FAP alpha dimer enzyme effective to down-regulate an abnormal immune response in the vicinity of the material surface. This may entail preventing the migration, accumulation or activation of immune cells in the vicinity of the material surface.

A subject shall mean a human or animal including but not limited to a dog, cat, horse, cow, pig, sheep, goat, chicken, rodent e.g., rats and mice, primate, e.g., monkey, and fish or aquaculture species such as fin fish (e.g., salmon) and shellfish (e.g., shrimp and scallops). Subjects suitable for therapeutic or prophylactic methods include vertebrate and invertebrate species. Subjects can be house pets (e.g., dogs, cats, fish, etc.), agricultural stock animals (e.g., cows, horses, pigs, chickens, etc.), laboratory animals (e.g., mice, rats, rabbits, etc.), zoo animals (e.g., lions, giraffes, etc.), but are not so limited. Although many of the embodiments described herein relate to human disorders, the invention is also useful for treating other nonhuman vertebrates.

The compositions, as described above, are administered in effective amounts. The effective amount will depend upon the mode of administration, the particular condition being treated and the desired outcome. It will also depend upon, as discussed above, the stage of the condition, the age and physical condition of the subject, the nature of concurrent therapy, if any, and like factors well known to the medical practitioner. For therapeutic applications, it is that amount sufficient to achieve a medically desirable result. In some cases an effective amount is that amount that down-regulates an immune response. Down-regulation of an immune response can be assessed in a number of ways. These include measuring white blood cell counts either locally or systemically (including neutrophil, macrophage and T cell counts), body temperature of the subject (e.g., presence or absence of a bodily temperature over 37.5°C, levels of cytokines or immunomodulators in a subject, swelling, pain, joint flexibility, range of motion, and the like. In some cases, IL-1 levels in a subject may be measured as an indicator of immune response down-modulation. Effective amounts may reduce IL-1 levels to a normal level or to a below normal level. A normal level of IL-1 is the level of IL-1 in a subject that is not experiencing an IL-1 mediated condition or any other condition that would impact upon IL-1 levels. Normal IL-1 levels in human serum are less than 4 pg/ml for both IL-1 alpha and IL-1 beta, individually. Cell and cytokine or mediator

levels may be measured in a bodily fluid from a subject including but not limited to blood, serum, plasma and cerebrospinal fluid.

Generally, doses of active compounds of the present invention would be from about 0.01 mg/kg per day to 1000 mg/kg per day. It is expected that doses ranging from 50-500 mg/kg will be suitable.

The methods of the invention, generally speaking, may be practiced using any mode of administration that is medically acceptable, meaning any mode that produces effective levels of the active compounds without causing clinically unacceptable adverse effects. A variety of administration routes are available including but not limited to oral, rectal, topical, nasal, intradermal, or parenteral routes. The term "parenteral" includes subcutaneous, intravenous, intramuscular, or infusion. Intravenous or intramuscular routes are not particularly suitable for long-term therapy and prophylaxis. They could, however, be preferred in emergency situations, such as for example in a sepsis situation. Generally, administration by injection is preferred.

When peptides are used therapeutically, in certain embodiments one desirable route of administration is by pulmonary aerosol. Techniques for preparing aerosol delivery systems containing peptides are well known to those of skill in the art. Generally, such systems should utilize components which will not significantly impair the biological properties of the antibodies, such as the paratope binding capacity (see, for example, Sciarra and Cutie, "Aerosols," in Remington's Pharmaceutical Sciences, 18th edition, 1990, pp 1694-1712; incorporated by reference). Those of skill in the art can readily determine the various parameters and conditions for producing protein or peptide aerosols without resort to undue experimentation.

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, anti-infectives, anti-oxidants, chelating agents, and

inert gases and the like. Lower doses will result from other forms of administration, such as intravenous administration. In the event that a response in a subject is insufficient at the initial doses applied, higher doses (or effectively higher doses by a different, more localized delivery route) may be employed to the extent that patient tolerance permits. Multiple doses per day are contemplated to achieve appropriate systemic levels of compounds.

The agents may be combined, optionally, with a pharmaceutically-acceptable carrier. The term "pharmaceutically-acceptable carrier" as used herein means one or more compatible solid or liquid filler, diluents or encapsulating substances which are suitable for administration into a human. The term "carrier" denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application. The components of the pharmaceutical compositions also are capable of being co-mingled with the molecules of the present invention, and with each other, in a manner such that there is no interaction which would substantially impair the desired pharmaceutical efficacy.

The invention in other aspects includes pharmaceutical compositions of the agents. When administered, the pharmaceutical preparations of the invention are applied in pharmaceutically-acceptable amounts and in pharmaceutically-acceptably compositions. Such preparations may routinely contain salt, buffering agents, preservatives, compatible carriers, and optionally other therapeutic agents. When used in medicine, the salts should be pharmaceutically acceptable, but non-pharmaceutically acceptable salts may conveniently be used to prepare pharmaceutically-acceptable salts thereof and are not excluded from the scope of the invention. Such pharmacologically and pharmaceutically-acceptable salts include, but are not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulfuric, nitric, phosphoric, maleic, acetic, salicylic, citric, formic, malonic, succinic, and the like. Also, pharmaceutically-acceptable salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts.

Various techniques may be employed for introducing nucleic acids of the invention into cells, depending on whether the nucleic acids are introduced in vitro or in vivo in a host. Such techniques include transfection of nucleic acid-$CaPO_4$ precipitates, transfection of nucleic acids associated with DEAE, transfection with a retrovirus including the nucleic acid of interest, liposome mediated transfection, and the like. For certain uses, it is preferred to target the nucleic acid to particular cells. In such instances, a vehicle used for delivering a nucleic acid of the invention into a cell (e.g., a retrovirus, or other virus; a liposome) can have

a targeting molecule attached thereto. For example, a molecule such as an antibody specific for a surface membrane protein on the target cell or a ligand for a receptor on the target cell can be bound to or incorporated within the nucleic acid delivery vehicle. For example, where liposomes are employed to deliver the nucleic acids of the invention, proteins which bind to a surface membrane protein associated with endocytosis may be incorporated into the liposome formulation for targeting and/or to facilitate uptake. Such proteins include capsid proteins or fragments thereof tropic for a particular cell type, antibodies for proteins which undergo internalization in cycling, proteins that target intracellular localization and enhance intracellular half life, and the like. Polymeric delivery systems also have been used successfully to deliver nucleic acids into cells, as is known by those skilled in the art. Such systems even permit oral delivery of nucleic acids.

Other delivery systems can include time-release, delayed release or sustained release delivery systems. Such systems can avoid repeated administrations of the FAP alpha dimer enzyme, increasing convenience to the subject and the physician. Many types of release delivery systems are available and known to those of ordinary skill in the art. They include polymer base systems such as poly(lactide-glycolide), copolyoxalates, polycaprolactones, polyesteramides, polyorthoesters, polyhydroxybutyric acid, and polyanhydrides. Microcapsules of the foregoing polymers containing drugs are described in, for example, U.S. Patent 5,075,109. Delivery systems also include non-polymer systems that are: lipids including sterols such as cholesterol, cholesterol esters and fatty acids or neutral fats such as mono- di- and tri-glycerides; hydrogel release systems; sylastic systems; peptide based systems; wax coatings; compressed tablets using conventional binders and excipients; partially fused implants; and the like. Specific examples include, but are not limited to: (a) erosional systems in which the anti-inflammatory agent is contained in a form within a matrix such as those described in U.S. Patent Nos. 4,452,775, 4,667,014, 4,748,034 and 5,239,660 and (b) diffusional systems in which an active component permeates at a controlled rate from a polymer such as described in U.S. Patent Nos. 3,832,253, and 3,854,480.

A preferred delivery system of the invention is a colloidal dispersion system. Colloidal dispersion systems include lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. A preferred colloidal system of the invention is a liposome. Liposomes are artificial membrane vessels which are useful as a delivery vector in vivo or in vitro. It has been shown that large unilamellar vessels (LUV), which range in size

from 0.2-4.0 μm can encapsulate large macromolecules. RNA, DNA, and intact virions can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form (Fraley, et al., *Trends Biochem. Sci.*, (1981) 6:77). In order for a liposome to be an efficient gene transfer vector, one or more of the following characteristics should be present: (1) encapsulation of the gene of interest at high efficiency with retention of biological activity; (2) preferential and substantial binding to a target cell in comparison to non-target cells; (3) delivery of the aqueous contents of the vesicle to the target cell cytoplasm at high efficiency; and (4) accurate and effective expression of genetic information.

Liposomes may be targeted to a particular tissue by coupling the liposome to a specific ligand such as a monoclonal antibody, sugar, glycolipid, or protein. Liposomes are commercially available from Gibco BRL, for example, as LIPOFECTIN™ and LIPOFECTACE™, which are formed of cationic lipids such as N-[1-(2, 3 dioleyloxy)-propyl]-N, N, N-trimethylammonium chloride (DOTMA) and dimethyl dioctadecylammonium bromide (DDAB). Methods for making liposomes are well known in the art and have been described in many publications. Liposomes also have been reviewed by Gregoriadis, G. in *Trends in Biotechnology*, (1985) 3:235-241.

In one important embodiment, the preferred vehicle is a biocompatible microparticle or implant that is suitable for implantation into the mammalian recipient. Exemplary bioerodible implants that are useful in accordance with this method are described in PCT International application no. PCT/US/03307 (Publication No. WO 95/24929, entitled "Polymeric Gene Delivery System"). PCT/US/03307 describes a biocompatible, preferably biodegradable polymeric matrix for containing an exogenous gene under the control of an appropriate promoter. The polymeric matrix is used to achieve sustained release of the exogenous gene in the patient. In accordance with the instant invention, the fugetactic agents described herein are encapsulated or dispersed within the biocompatible, preferably biodegradable polymeric matrix disclosed in PCT/US/03307.

The polymeric matrix preferably is in the form of a microparticle such as a microsphere (wherein an agent is dispersed throughout a solid polymeric matrix) or a microcapsule (wherein an agent is stored in the core of a polymeric shell). Other forms of the polymeric matrix for containing an agent include films, coatings, gels, implants, and stents. The size and composition of the polymeric matrix device is selected to result in favorable release kinetics in the tissue into which the matrix is introduced. The size of the polymeric

matrix further is selected according to the method of delivery which is to be used. Preferably when an aerosol route is used the polymeric matrix and agent are encompassed in a surfactant vehicle. The polymeric matrix composition can be selected to have both favorable degradation rates and also to be formed of a material which is bioadhesive, to further increase the effectiveness of transfer. The matrix composition also can be selected not to degrade, but rather, to release by diffusion over an extended period of time.

In another important embodiment the delivery system is a biocompatible microsphere that is suitable for local, site-specific delivery. Such microspheres are disclosed in Chickering et al., *Biotech. And Bioeng.*, (1996) 52:96-101 and Mathiowitz et al., *Nature*, (1997) 386:.410-414.

Both non-biodegradable and biodegradable polymeric matrices can be used to deliver the agents of the invention to the subject. Biodegradable matrices are preferred. Such polymers may be natural or synthetic polymers. Synthetic polymers are preferred. The polymer is selected based on the period of time over which release is desired, generally in the order of a few hours to a year or longer. Typically, release over a period ranging from between a few hours and three to twelve months is most desirable. The polymer optionally is in the form of a hydrogel that can absorb up to about 90% of its weight in water and further, optionally is cross-linked with multivalent ions or other polymers.

In general, agents are delivered using a bioerodible implant by way of diffusion, or more preferably, by degradation of the polymeric matrix. Exemplary synthetic polymers which can be used to form the biodegradable delivery system include: polyamides, polycarbonates, polyalkylenes, polyalkylene glycols, polyalkylene oxides, polyalkylene terepthalates, polyvinyl alcohols, polyvinyl ethers, polyvinyl esters, poly-vinyl halides, polyvinylpyrrolidone, polyglycolides, polysiloxanes, polyurethanes and co-polymers thereof, alkyl cellulose, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, nitro celluloses, polymers of acrylic and methacrylic esters, methyl cellulose, ethyl cellulose, hydroxypropyl cellulose, hydroxy-propyl methyl cellulose, hydroxybutyl methyl cellulose, cellulose acetate, cellulose propionate, cellulose acetate butyrate, cellulose acetate phthalate, carboxylethyl cellulose, cellulose triacetate, cellulose sulphate sodium salt, poly(methyl methacrylate), poly(ethyl methacrylate), poly(butylmethacrylate), poly(isobutyl methacrylate), poly(hexylmethacrylate), poly(isodecyl methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate),

poly(octadecyl acrylate), polyethylene, polypropylene, poly(ethylene glycol), poly(ethylene oxide), poly(ethylene terephthalate), poly(vinyl alcohols), polyvinyl acetate, poly vinyl chloride, polystyrene, polyvinylpyrrolidone, and polymers of lactic acid and glycolic acid, polyanhydrides, poly(ortho)esters, poly(butiric acid), poly(valeric acid), and poly(lactide-cocaprolactone), and natural polymers such as alginate and other polysaccharides including dextran and cellulose, collagen, chemical derivatives thereof (substitutions, additions of chemical groups, for example, alkyl, alkylene, hydroxylations, oxidations, and other modifications routinely made by those skilled in the art), albumin and other hydrophilic proteins, zein and other prolamines and hydrophobic proteins, copolymers and mixtures thereof. In general, these materials degrade either by enzymatic hydrolysis or exposure to water in vivo, by surface or bulk erosion.

Examples of non-biodegradable polymers include ethylene vinyl acetate, poly(meth)acrylic acid, polyamides, copolymers and mixtures thereof.

Bioadhesive polymers of particular interest include bioerodible hydrogels described by H.S. Sawhney, C.P. Pathak and J.A. Hubell in *Macromolecules*, (1993) 26:581-587, the teachings of which are incorporated herein, polyhyaluronic acids, casein, gelatin, glutin, polyanhydrides, polyacrylic acid, alginate, chitosan, poly(methyl methacrylates), poly(ethyl methacrylates), poly(butylmethacrylate), poly(isobutyl methacrylate), poly(hexylmethacrylate), poly(isodecyl methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), and poly(octadecyl acrylate).

In addition, important embodiments of the invention include pump-based hardware delivery systems, some of which are adapted for implantation. Such implantable pumps include controlled-release microchips. A preferred controlled-release microchip is described in Santini, JT Jr., et al., *Nature*, 1999, 397:335-338, the contents of which are expressly incorporated herein by reference.

Use of a long-term sustained release implant may be particularly suitable for treatment of chronic conditions. Long-term release, as used herein, means that the implant is constructed and arranged to delivery therapeutic levels of the active ingredient for at least 30 days, and preferably 60 days. Long-term sustained release implants are well-known to those of ordinary skill in the art and include some of the release systems described above.

As discussed above, in certain embodiments, the agents of the invention are delivered directly to the site at which there is inflammation, e.g., the joints in the case of a subject with rheumatoid arthritis, the blood vessels of an atherosclerotic organ, etc. For example, this can be accomplished by attaching an agent (nucleic acid or polypeptide) to the surface of a balloon catheter; inserting the catheter into the subject until the balloon portion is located at the site of inflammation, e.g. an atherosclerotic vessel, and inflating the balloon to contact the balloon surface with the vessel wall at the site of the occlusion. In this manner, the compositions can be targeted locally to particular inflammatory sites to modulate immune cell migration to these sites. In another example the local administration involves an implantable pump to the site in need of such treatment. Preferred pumps are as described above. In a further example, when the treatment of an abscess is involved, the agent may be delivered topically, e.g., in an ointment/dermal formulation. Optionally, the agents are delivered in combination with other therapeutic agents (e.g., anti-inflammatory agents, immunosuppressant agents, etc.).

The invention will be more fully understood by reference to the following examples. These examples, however, are merely intended to illustrate the embodiments of the invention and are not to be construed to limit the scope of the invention.

Examples

Abbreviations:

The following abbreviations are used throughout the specification and claims and in the Examples:

"a.a.": amino acid.

Amino acid single letter code: A=Alanine; C=cysteine; D=aspartic acid; E=glutamic acid; F=phenylalanine; G=glycine; H=histidine; I=isoleucine; K=lysine; L=leucine; M=methionine; N=asparagines; P=proline; Q=glutamine; R=arginine; S=serine; T=threonine; V=valine; W=tryptophan; Y=tyrosine.

Amino acid mutations: e.g. A657D denoted alanine residue 657 mutated to aspartate.

"Nitrilo- derivative": A class of Xaa-proline dipeptide inhibitors characterized by replacement of the carboxyl group of the proline with the nitrilo (cyano) group. Xaa = any amino acid.

"Overlap extension PCR": Overlap extension PCR is a polymerase chain reaction (PCR) based technique for insertion of mutations or restriction sites at any point in a DNA molecule (Kadowaki et al. 1989. Gene. 76, 161-166). It proceeds in two successive rounds of PCR. Typically, in the first round, two separate PCR reactions (Tube A and Tube B) are run. In each, one of a pair of divergent overlapping mutagenic primers is paired with an external non-mutagenic primer to give overlapping PCR products with the mutation at their 3' in one case and 5' end in the other. The mutagenic primers are designed to ensure productive annealing to the template on one hand, typically with 6-20 perfectly matched nucleotides 3' of the mutation(s), and with each other on the other. The two PCR products from the first round are then mixed and PCR repeated with only the external primers. The overlap near the mutagenic site allows the products to anneal and so prime each other, so that after fill-in by the thermostable polymerase, they become a single long product. The latter is then amplified in the same reaction by the external primers giving a product that can then be cut using available restriction sites. These flanking restriction sites can be any reasonable distance away from the site of mutation and their availability determines the location of the external primers. The resultant fragment is then used to replace the corresponding wild-type fragment, yielding the desired mutation.

"Primer": An oligonucleotide capable of annealing to a specified DNA target and serving as a priming site for DNA polymerase activity.

"RT-PCR". Polymerase chain reaction on cDNA derived from RNA via reverse transcription.

"sr hFAP": soluble recombinant human FAP

"sr hDPPIV": soluble recombinant human DPPIV.

"Val-boroPro": L-valine-pyrollidine-2-boronic acid dipeptide inhibitor.

Example 1.1:

Normal B6 mice (+/+) and congenic B6.129/s7-Il1r1tm1Imx mice with a targeted mutation of the IL-1 receptor-1 (-/-) were orally administered 160-μg PT-100 or saline. Eight hours after PT-100 administration, the levels of cytokines and chemokines indicated on the ordinates were determined by ELISA of serum or spleen protein extracts for IL-1 beta (FIG. 3). Data represent the increases observed in PT-100 treated mice after correction for control levels in saline-treated mice. IL-1 beta levels were normalized so as to correct for differences between the total protein concentrations in extracts. The data indicates that loss of the IL-1 beta receptor results in loss of production of other cytokines except IL-1 beta itself.

Example 1.2:

BALB/c *CD26*$^{+/+}$ wild-type and BALB/c CD26$^{-/-}$ mice were treated with various doses of PT-100 and the resulting cytokine and chemokine profile of these mice was analyzed. Dose response curves for the indicated cytokine or chemokine are shown in FIG. 4. Data represent the mean ±SE (n=5) responses determined by ELISA of serum samples. CD26 knockout mice have a strong cytokine response to PT100 despite the loss of CD26, indicating that another dipeptidyl peptidase, likely FAP, is responsible for the observed response.

Example 1.3:

Stromal cells from humans and Fischer D^{-} (CD26 mutant) rats were isolated and treated in vitro with PT-100. Stromal cells were incubated in multiwell plates with or without the addition of PT-100 for 4 (human cells) or 8 (rat cells) hours. IL-1 beta levels in supernatants were determined by ELISA. Data represent the means of duplicate cultures for each experiment. The levels of IL-1 beta in tissue culture supernatants of these cells after several hours of incubation are increased as shown in Table 1, indicating that CD26 is not essential to the IL-1 response induced by PT-100 administration.

Table 1. Stimulation of IL-1 beta production by PT-100 in human bone marrow stromal cell cultures

Source of stromal cells	Experiment No.	IL-1 beta concentration (pg/ml) after incubation with[1]:

		Medium	10 μM PT-100
Human	1	0.0	201.0
	2	0.0	36.0
	3	1.2	19.2
Fischer D⁻ rat	4	0.0	79.0

Example 1.4: Production of soluble recombinant human FAP:

This strategy is based on information on the N-terminus of serum DPPIV (Durinx et al. Eur J Biochem. 2000 Sep;267(17):5608-13). A truncated FAP was engineered in which a signal/leader sequence was joined to the residue in FAP analogous to the N-terminus of serum DPPIV to allow secretion.

The cDNA encoding the desired truncated human FAP alpha dimer enzyme is engineered into the mammalian secretion vector pSecTag2 (Cat. # V900-20, InVitrogen Corporation). The vector, available in A, B or C versions, representing three possible phases for gene fusion, contains an immunoglobulin-kappa light chain secretion signal followed by a selection of restriction sites for gene insertion. The fusion requires engineering a restriction site upstream of the chosen fusion amino acid in the 5' end of the FAP alpha dimer enzyme nucleic acid in phase with the chosen restriction site (Sfi I) in the vector secretion sequence. The chosen fusion amino acid in the 5' end of the FAP (Thr38) is 3' of the trans-membrane anchoring domain. The pSecTag2 version B and its Sfi I restriction site are chosen for the fusion because it minimizes the additional N-terminal, vector-encoded residues in the mature secreted protein.

Construction of the fusion is in 3 stages. First, human FAP alpha dimer enzyme cDNA corresponding to nucleotide 161-2526 approximately of wild type FAP alpha dimer enzyme (GenBank Accession number NM_004460) is obtained by reverse transciptase then Taq DNA polymerase mediated PCR (RT-PCR) on RNA from human stromal cells grown from bone marrow, and inserted into vector pPCR2.1 (InVitrogen Corporation) using the T/A cloning method, giving a plasmid pTAhFAP#2. The primers for this are hFAP1 (5' ccacgctctg aagacagaat tagc 3' SEQ ID NO: 11) and hFAP2 (5' tcagattctg atagaggctt gc 3' SEQ ID NO: 12). Next, the cloned FAP cDNA, characterized as containing a single point mutation T229M, is excised with flanking BamHI and Not I restriction enzymes (contributed

by the cloning vector), and inserted into similarly-cut pSecTag2-B vector, and which gives the correct orientation relative to the secretion signal but retains the complete FAP coding sequence plus untranslated upstream sequence (plasmid #13).

Completion of the final plasmid requires deletion of the first 37 amino acids of FAP and insertion of Sfi I restriction site upstream of the Thr38 codon to allow an in-phase junction to the Ig-kappa secretion sequence at the Sfi I site. The 5' PCR primer sequence for insertion of the Sfi I site is chosen so that the same primer served for both human and mouse constructs. This makes residue #40 a lysine, as found in mouse wild type FAP alpha dimer enzyme. It is to be understood that the invention also embraces soluble hFAP having a methionine at residue #40 (as in wild type hFAP). This 5' primer, named Sfi-FAP-B, had the sequence 5' GTAGTCGGCC CAGCCGGCCA CAAAGAGAGC TCTTACCCTG AAGGATATTT TAAATG 3', SEQ ID NO: 13 (Sfi I site underlined). PCR of FAP cDNA with this primer and a reverse primer located 3' of the unique Xba I site (located in the codons for amino acid 113-115), gives a PCR product of approx. 700 nt. The Sfi I-Xba I double digest on plasmid #18 is used to remove the native 5' end of FAP up to the internal Xba I site at codon 114 and to cut the FAP PCR product. The appropriate fragments of >5 kb and approx. 259 nt respectively are isolated from an agarose gel using standard procedures (known to those skilled in the art) and ligated to each other. After transformation into bacteria and screening of colonies, those with correct properties are sequenced to ensure the correct fusion junction and absence of PCR-induced mutations, giving plasmid #122 which is designated wild-type FAP. The N-terminus of the final mature amino acid sequence of cleaved secreted product will contain 6 amino acids from the vector, DAAQPA, SEQ ID NO: 14 , fused to the truncated FAP sequence, of which the first 13 amino acids are TKRALTLKDILNG, SEQ ID NO: 15 . FIG. 10 demonstrates soluble FAP and DPPIV activity in several harvests of tissue culture supernatant from plasmids #122 and #135 respectively. Two amounts of plasmid (10 micrograms and 20 micrograms) were used to transfect HEK293T cells in a 10 cm diameter dish and harvests taken at 23.5, 39.5, 51.5 and 62h after addition of DNA to the cells. Assays contained 100 microlitre 50mM HEPES/NaOH buffer pH 8.1, 1mM (#122) or 0.1 mM (#135) Ala-Pro-AFC substrate and 11 microlitres tissue culture supernatant. Assays were incubated at 37C and stopped after 1.75 h with 100 microlitres 1M sodium acetate pH4.5 and fluorescence read at 505 nm (Excitation at 400 nm).

Published sequence of N-terminus of serum hDPPIV and inferred analogous site for FAP:

```
hDPPIV: MKTPWKVLLGLLGAAALVTIITVPVVLLNKGTDDATADSRKTYTLTDYLKN-- (SEQ ID NO: 16)
Serum DPP4*:                                    SRKTYTLTDYLKN-- (SEQ ID NO: 17)
                                                RKTYTLTDYLKN--(SEQ ID NO: 18)
                                                 :..|| | |.:
hFAP:     MKTWVKIVFGVATSAVLALLVMCIVLRPSRVHNSEENTMRALTLKDILNG-- (SEQ ID NO: 19)
Proposed N-terminus:                            TMRALTLKDILNG-- (SEQ ID NO: 20)
mFAP:     MKTWLKTVFGVTTLAALALVVICIVLRPSRVYKPEGNTKRALTLKDILNG-- (SEQ ID NO: 21)
Proposed N-terminus:                            TKRALTLKDILNG-- (SEQ ID NO: 22)
```

pSecTag2 vector (InVitrogen):

```
                                                                    SfiI
932  GTA CTG CTG CTC TGG GTT CCA GGT TCC ACT GGT GAC GCG GCC CAGCCG
     Val Leu Leu Leu Trp Val Pro Gly Ser Thr Gly Asp
                                    Signal cleavage site
```

(SEQ ID NO: 23 for nucleotide sequence, and SEQ ID NO: 24 for amino acid sequence)

DNA sequence of vector-FAP junction showing the 6 vector-derived amino acids included in the mature secreted protein:

```
          SfiI
5'ggt/gacgcggcccagccggcc ACAAAGAGAGCTCTTACCcTGAAGGATATTTTAAATG3' (SEQ ID NO: 65)
  g  /D  A  A  Q  P  A  -T  K  R  A  L  T  L  K  D  I  L  N  G  (SEQ ID NO: 25)
     /-------Vector-----|-----FAP--- >
```

Example 1.5: Preparation of soluble FAP alpha dimer enzyme:

DNA of the FAP alpha dimer enzyme containing plasmid is prepared on a approximately 400 µg scale from overnight 30 ml cultures in Luria broth with 100 µg ampicillin per ml using a commercial kit (Qiagen Maxiprep Kit). Ten (10) µg of DNA and 30 µl of Lipofectamine 2000 transfection reagent (InVitrogen Corporation) are used to transiently transfect 293T cells in 10 cm diameter tissue culture plates using the manufacturer's protocol. Cells are at greater than about 70% confluent in Freestyle 293 Expression Medium (InVitrogen Corporation) containing 2.5% fetal calf serum and standard antibiotics penicillin and streptomycin. Antibiotic-free medium is used for the initial 18-24 h

of transfection, after which serum-free medium with antibiotics is employed. Culture supernatant containing the secreted recombinant enzyme is harvested 6-18 h later and again 24 h after addition of fresh serum-free medium and is stored in a cold room. FIG. 9 and FIG. 10 show FAP activity in several harvests of tissue culture supernatant from various plasmids expressing secreted soluble FAP alpha dimer enzyme.

Example 1.6: Assay of soluble FAP alpha dimer enzyme:

A typical activity assay consists of 135 µl 50 mM HEPES/Na buffer pH 8.1 (or other pH), 140 mM NaCl, 10-15 µl enzyme-containing culture supernatant, dipeptide substrate Ala-Pro-(7-amino-4-trifluoromethyl coumarin) (abbreviated Ala-Pro-AFC) at typically 0.25-1 mM (unless Km determinations require variation) added from a 100 or 400 mM stock in dimethyl formamide. Other buffers can be substituted. Assays lacking either substrate or enzyme are set up in 96-well microtitre plates, pre-warmed at the desired incubation temperature between room temperature (22°C) to 37°C. Then the missing component is added to start the reaction and incubation continued at the desired temperature. Production of the fluorescent AFC product is either monitored continuously in a thermostatted fluorometer or after termination with one to one-tenth volume 1 M sodium acetate pH 4.5.

Example 1.7: Immunoprecipitation results with FAP-specific mAb:

Soluble FAP alpha dimer enzyme was isolated from the supernatant according to two methods: capture on Protein G beads and capture on Protein G coated 96-well plates. These approaches are discussed below.

The protocol for capture on Protein G beads is as follows:

Tris buffer / NaCl / 1% triton	100 µl
Anti-FAP mAb supernatant	100 µl
Soluble FAP supernatant	300 µl-1 ml

Supernatant containing FAP alpha dimer enzyme was incubated with anti-FAP mAb (i.e., tissue culture medium from F19 anti-hFAP hybridoma) for 20 min on ice. Then 38 µl Protein G beads (50% v/v) were added and tubes rocked 1 hr at 4°C. Beads were washed 2X with Triton-containing buffer, then once with 600 µl 50 mM HEPES pH 8.1, 140 mM NaCl. Finally, beads were suspended in 500 ul 50 mM HEPES pH 8.1, 140 mM NaCl containing

100 μM Ala-Pro-AFC substrate, incubated at 37°C in a rocker for 10 min to 2.5 h, stopped with 0.1-1 vol 1 M NaOAc pH 4.5 and centrifuged to pellet beads.

The protocol for capture on Protein G coated 96 well plates is as follows:

100 μl goat anti-mouse IgG (H+L) polyclonal antibody was captured in wells of Protein G coated 96 well plates (PIERCE Biotechnology) by incubating at room temperature for 1 h at room temp. Wells were then washed and to them was added 100 μl anti-hFAP hybridoma supernatant for 1 h at room temp. Unbound mAb was washed away and 100 μl cell extract / 293T supernatant containing FAP alpha dimer enzyme was added. The wells were incubated 1 h at 4°C, and then washed twice with Triton-containing buffer, and once with 50 mM HEPES pH 8.1, 140 mM NaCl. To the wells were added 90 μl of 50 mM HEPES pH 8.1, 140 mM NaCl were added, followed by addition of inhibitor PT-100 (10 μl of 10X), if needed, and incubation at room temp for 15-20 min, if inhibitor was used. Then, 10 μl Ala-Pro-AFC substrate (11x) was added giving 100 μM final concentration. Plates were incubated at 37°C for 40 min, stopped with 1 vol 1 M NaOAc pH 4.5 and the fluorescence read at 505 nm with excitation at 400 nm in a Molecular Dynamics Spectra Max GeminiXS Fluorescence plate reader. Activity of immunoprecipitated recombinant soluble human FAP is shown in FIGs. 6 and 8.

Example 1.8: Inhibition of soluble recombinant human FAP by PT-100:

Assays are done in a dark-sided 96-well plate. PT100 stock (0.1 M in 0.1 M HCl) was thawed, diluted in assay buffer (50 mM HEPES pH 8.1, 140 mM NaCl) immediately before use and added to enzyme.

The reaction conditions were as follows:

50 mM HEPES pH 8.1, 140 mM NaCl	160 ul
Soluble FAP (culture supernatant)	10-20 ul
Inhibitor (diluted to 20X)	10 ul

The enzyme was incubated for 10-20 min at room temp with the PT-100 in order to provide sufficient time for PT-100 to bind. The solution was then warmed 5-10 min at 37°C, following with 20 μl of 2.5 mM or 1 mM Ala-Pro-AFC substrate (10 mM stock is diluted in DMF by 10X to give final concentration of 0.1 mM) was added. The solution was incubated at 37°C for 20 min to1 h. The reaction was stopped with 0.1-1 vol of 1 M NaOAc pH 4.5.

Fluorescence was read at 505 nm with excitation at 400 nm in a Molecular Dynamics Spectra Max GeminiXS Fluorescence plate reader.

Wild type FAP alpha dimer enzyme was immunoprecipitated from Triton X100 extracts of RPMI-7951 cells and soluble FAP alpha dimer enzyme was immunoprecipitated from supernatant of transiently transfected 293T cells using Protein G coated 96 well plates as described above. Assays in the presence of PT-100 were done as described above. The inhibitor assay was performed as described above. The results of this assay are shown in FIG. 8.

Conclusions:

Human and mouse FAP can be produced in a recombinant soluble secreted form, as shown in FIGs. 5, 9 and 10. The recombinant soluble human FAP however is recognized by mouse-derived hFAP specific mAb F19, while the murine version is not. This indicates that the cloning procedure did not affect the epitope structure of either FAP form, as relates to the mAb F19. This is shown in FIG. 6.

PT-100 inhibits recombinant hFAP in 293T supernatants with IC_{50} ~20 nM, as shown in FIG. 7. There is no difference in IC_{50} between immunocaptured FAP from a native source or produced from the recombinant plasmid, as shown in FIG. 8.

Example 2: Expression and secretion of soluble recombinant human FAP alpha dimer enzyme starting at codon #38 using a secretion sequence derived from the cytokine G-CSF:

A plasmid is constructed containing a portion of wild type FAP alpha dimer enzyme fused in phase to a functional human G-CSF leader sequence using the overlap extension PCR technique. Round 1, Tube A: G-CSF leader is obtained by PCR from cDNA from 15-24 h LPS-treated human bone marrow stromal cells using primers hG-CSF F (5' CCAAGCTG GCTAGC CACCATG gctggac ctgccacccagag, SEQ ID NO: 26) and hG-CSF leader-R (5' GGC TTC CTG CAC TGT CCA GAG TGC ACT 3', SEQ ID NO: 27). Round 1 Tube B: The human FAP 5' end is amplified with primers hG-CSF_FAP-F (5' GCACTCTGGA CAGTGCAGGA AGCC ACAAAG AGAGCTCTTA CCcTGAAGGA TATTTTA 3', SEQ ID NO: 28) and any primer 3' of the XbaI site such as hFAP-ClaI-R (5' GCA GGG TAA GTG GTA TCG ATA ATA AAT ATC CG 3', SEQ ID NO: 29). Round 2 mixes the 2 Round

1 PCR products with the flanking hG-CSF-F and hFAP-ClaI-R primers, followed by NheI and XbaI (or ClaI) digestion and replacement of the corresponding piece in plasmid #122, #13 or #23. (See Examples 1.4 and 5.3 for origins of these numbered plasmids.)

Example 3: Expression and secretion of soluble recombinant human DPPIV containing a 6 amino acid N-terminal extension in a mammalian cell line:

Total RNA is isolated from the Caco-2 colorectal carcinoma cell line (ATCC HTB-37) by standard Trizol/phenol/chloroform methodology. The purified RNA (approx. 2.5 μg in a 20 μl reaction) is used to make cDNA using oligo-dT primer and a commercial reverse Transcriptase (RT) kit (InVitrogen). An aliquot (2 microlitre) of the RT reaction is used to PCR amplify a truncated coding region of DPPIV starting at S39 with primers Sfi-DPPIV (5' GTAGTCGGCC CAGCCGGCC AGTCGCAAAA CTTACACTCT AACTGATTAC TTAAAAAAT 3', SEQ ID NO: 30) and primer DPP4-R 5' gtcggagcgg ccgcctaagg taaagagaaa cattgtttta tgaagtg 3' (SEQ ID NO: 31) with program 94°C for 45 sec initial denaturation, then 30 cycles of 94°C, 10 sec; 48°C, 6 sec; 60°C, 4 min; followed by a 5 min extension at 72°C after cycling. The resultant PCR product is cleaved with restriction enzymes SfiI for 25 min at 50°C, then 1 hr with NotI at 37°C. The approx. 2.2 kb fragment is then inserted into pSecTag2-B vector cut with same, and transformed into bacteria under standard conditions. The resulting plasmid is #135.

Example 4.1: Mutation of FAP amino acid residue alanine 657 to aspartic acid:

Aspartate 663 in DPPIV is one of a number of residues identified that is close to the valine and peptide bond of the bound inhibitor in the published crystal structure of DPPIV. It differs from the corresponding residue in wild type FAP alpha dimer enzyme (Ala 657 in FAP) suggesting that it is not critical for catalytic activity in this class of enzyme. This putative active site residue in FAP is replaced with the corresponding aspartate residue from DPPIV (D663). Replacement of the identified residue is done using standard overlap extension PCR. PCR primers for A657D mutation are forward mutagenic internal 5' tccagctggg aatattacGA Ctctgtctac acagagagat t 3' (SEQ ID NO: 32); Reverse mutagenic internal: 5' AAT CTC TCT GTG TAG ACA GAG TCG TAA TAT TCC CAG CTG GA 3' (SEQ ID NO: 33); and two non-mutagenic flanking primers hFAP-RV-F (forward): 5' TAG ATG GAA ATT ACT TAT GGT ACA AGA TGA TTC TTC C 3' (SEQ ID NO: 34)

(located ca.100-120 nt upstream of unique EcoRV site near nt 1747-1782 of in hFAP sequence, Accession No. NM_004460); and hFAP-Not-R (reverse): 5' ggt cgc tca gcg gcc gct tagtc tga caa aga gaa aca ctg ctt tag 3' (SEQ ID NO: 35) (with NotI restriction site (underlined) placed immediately after the stop codon).

PCR mutagenesis is accomplished in two rounds: Round 1: Tube A: 25 µl 1X KOD XL buffer, 0.2 µl KOD XL enzyme (Novagen, Madison, WI), 0.5 µl 10 uM hFAP-RV-F primer, 0.5 µl 10 uM A657D reverse primer. The template is 0.5 µl 1:500 dilution of plasmid #122 containing recombinant soluble hFAP in pSecTag2-B vector (InVitrogen) (approx. 0.5 ng). Tube B: 25 µl 1X KOD XL buffer, 0.2 µl KOD XL enzyme (Novagen), 0.5 µl 10 µM hFAP-Not-R primer, 0.5 µl 10 µM A657D forward primer. The template is 0.5 µl 1:500 dilution of plasmid #122 recombinant soluble hFAP in pSecTag2-B vector (InVitrogen) (approx. 0.5 ng). Round 2: 50 µl 1X KOD buffer, 0.4 µl KOD XL enzyme (Novagen), 1 µl 10 µM hFAP-RV-F primer, 1 µl 10 µM hFAP-Not-R primer. The template is 0.5 µl each Round 1, Tube A and Tube B PCR reactions. Cycling parameters for both rounds are: initial denaturation at 94°C for 40 sec; then 25 cycles of 94°C for 15 sec, 54°C for 15 sec and 72°C for 1 min. After cycling, extension is continued at 72°C for 5 min followed by cooling to 4°C. PCR products are isolated using a commercial kit (Qiagen), cut with EcoRV and NotI restriction enzymes, run on an agarose gel, and the approx. 600 nt fragment isolated using a commercially available kit (Qiagen). The recovered fragment is ligated to similarly-cut FAP-pSecTag2-B plasmid, to replace the wild-type fragment with the corresponding mutated fragment. Ligated DNA (0.5 microlitre) is transformed into commercially available E. coli electrocompetent cells and plated with ampicillin selection on LB plates. DNA minipreparations on resulting transformants are subjected to DNA sequencing using a commercial service to confirm the intended mutation. Techniques for these standard molecular biology procedures are familiar to those skilled in the art, and alternative strategies and techniques exist for accomplishing the same end.

The resulting plasmid is named #233. DNA sequencing, large scale plasmid preparation and transfection of mammalian cells are done using standard procedures, and the cell culture supernatant containing the secreted enzyme is collected. Production and testing of the recombinant enzyme is by methods described herein.

The effect of the A657D mutation on selected properties of soluble FAP alpha dimer enzyme are shown in FIG. 12. FIG. 12A shows that the pH-activity profile is less sensitive to

pH and is similar to DPPIV as shown in FIG. 13A, compared to the control plasmid #122. The latter (plasmid #122) shows the typical FAP alpha dimer enzyme pH profile reported in the literature. FIG. 12B shows that FAP mutant #233 is inhibited less strongly than the wild-type control #122. FIG. 12C shows that the degree of inhibition by val-boroPro is altered in the A657D mutant to be dependent on when the inhibitor is added relative to the substrate. However, plasmid #122 FAP is relatively insensitive to pre-inhibition. Pre-incubation of enzyme #233 with PT100 (FIG. 12C) renders it more inhibited than when substrate and inhibitor are added simultaneously. This indicates that slow-binding is occurring in mutant #233 and furthermore, that the presence of substrate protects from inhibitor binding, since the rate with simultaneous addition does not fall over time to the inhibited level seen with pre-incubation, even after 10 min into the assay. FIG. 12D (plasmid #122 control) and FIG. 12E (A657D mutant, plasmid #233) show activity versus Ala-Pro-AFC substrate concentration for Km determination. The scales are different because the Km is significantly lower in the A657D mutant (16 microMolar) compared to 490 microMolar in the control FAP alpha dimer enzyme from plasmid #122. Published values for full length WT FAP range from 200-460 microMolar (Sun et al., 2002, Protein Expr. Purif. 24, 274-281). The Km of the A657D mutant is very similar to that of DPPIV (FIG. 13D).

Unexpectedly, several significant properties are altered by the single A657D mutation, including decreased Km for synthetic substrate, broader pH optimum, an altered IC50 as well as slow-binding kinetics of proline boronic acid inhibitor val-boroPro, acquisition of sensitivity to inhibitor L-valine-2-nitrilo pyrollidine, and apparent irreversibility of inhibition by Val-boroPro. These coincide with desired improvements in FAP properties.

Example 4.2: Mutation of hFAP residues Y124H and A207S:

The intended mutations are produced using overlap extension PCR. For Round 1 PCR Tube A, the 5' PCR primer (Y124H-F: 5' TTTGTATATC TAGAAAGTGA TTATTCAAAG CTTTGGAGAC ACTCTTACACA G 3', SEQ ID NO: 36) overlaps the XbaI site and also serves to change the nearby Y124 to histidine, and the 3' reverse primer (A207S-R: 5' CCA GAG AGC ATA TTT TGT AGA AAG CAT TTC CTC TTC (SEQ ID NO: 37) overlaps and mutates A207 to serine. Tube B PCR has the mutagenic A207S primer in the forward sense (A207S-F: 5'gaagaggaaatgcttTctacaaaatatgctctctgg 3', SEQ ID NO: 38) and a reverse primer 3' to the unique ClaI site.

The products of PCR with these primers, using standard conditions, are mixed (0.5 µl each in a 50 µl reaction) and Round 2 PCR done with just the outermost primers Y124H-F and hFAP-Cla-R. The resulting PCR product of approx. 480 nt is purified and cut with XbaI and ClaI, run on agarose and the band excised and DNA gel purified. This is then ligated to sr hFAP plasmid from which the corresponding XbaI –ClaI fragment had been excised. The resulting plasmid is named #217. DNA sequencing, large scale plasmid preparation and transfection of mammalian cells are done using standard procedures, and the cell culture supernatant containing the secreted enzyme is collected.

Example 4.3: Mutation of hFAP residues A347V, G349R, F351R and V352P:

The intended mutations are produced using overlap extension PCR, using standard conditions. For Round 1 PCR Tube A, the 5' forward primer (hFAP-Cla-F 5' CGG ATA TTT ATT ATC GAT ACC ACT TAC CCT GC 3', SEQ ID NO: 39) is paired with the mutagenic R356-R. primer (5' TGA AGG CCT AAA TCT TCC AAC CCA Tcc agt tct gct ttc ttc tat atgctcc 3', SEQ ID NO: 40). For Tube B the 5' PCR primer (R356-F: 5' TGGGTTGGAA GATTTAGGCC Ttcaacacc agttttcag ctatgatg 3', SEQ ID NO: 41) is combined with the 3' reverse primer hFAP-RV-R (5' ctgtatttgctgttaat tgG ATA TCttaccttgcaagcacagaaaacatt 3', SEQ ID NO: 42). The products of Round 1 PCR with these primers are mixed (0.5 µl each in a 50 µl reaction) and Round 2 PCR done with just the outermost primers hFAP-Cla-F and hFAP-RV-R. The resulting PCR product of approximately 910 nt is purified and cut with Cla I and EcoRV, run on agarose and the approximately 875 nt band excised and DNA gel purified. This is then ligated to sr hFAP plasmid from which the corresponding Cla I-EcoRV fragment has been excised.

The resulting plasmid is named #219. DNA sequencing, large scale plasmid preparation and transfection of mammalian cells are done using standard procedures, and the cell culture supernatant containing the secreted enzyme is collected.

Example 4.4: Recombinant hFAP with mutations Y124H, A207S, A347V, G349R, F351R and V352P:

Plasmids #217 and #219 from above examples are spliced together to combine the sets of mutations therein. The mutated Xba I – Cla I fragment from plasmid #217 is excised with the cognate enzymes and ligated to plasmid #219 from which the corresponding fragment has been excised. This gives a plasmid named #257. DNA sequencing, large scale plasmid

preparation and transfection of mammalian cells are done using standard procedures, and the cell culture supernatant containing the secreted enzyme is collected.

Example 4.5: Recombinant hFAP with mutations Q732E and N733D in a mouse/human FAP chimera:

This alters two residues N-terminal to the catalytic histidine. The intended mutations are produced using overlap extension PCR using plasmid #23 containing chimeric FAP with N-terminal 77 amino acids from mouse FAP and the remainder human FAP with T229M mutation as template. For Round 1 PCR Tube A, the 5' PCR primer (hFAP-RV-F 5' TAGATGGAAA TTACTTATGG TACAAGATGA TTCTTCC 3', SEQ ID NO: 43) pairs with the mutagenic 3' reverse primer (DEDH-R 5' aatgtggtac tctgacGaAG accacggctt atccggcctg t 3', SEQ ID NO: 44). Tube B PCR has the mutagenic (DEDH-F 5' tggtcttcgt cagagtacca cattgcctgg 3', SEQ ID NO: 45) primer in the forward sense and the reverse primer pSecTag-R (5' GGCGCTATTC AGATCCTCTT CTGAGAT 3', SEQ ID NO: 46). The products of PCR with these primers are mixed (0.5 µl each in a 50 µl reaction) and Round 2 PCR done with just the outermost primers. The resulting PCR product of approx. 760 nt is purified and cut with EcoRI and Not I, run on agarose and the approx. 250 nt gel band excised and DNA purified. This is then ligated to plasmid #23 (rs hFAP chimera with 77 N-terminal amino acids from mFAP) from which the corresponding EcoRI–Not I fragment has been excised. The resultant plasmid is designated #94 which is confirmed by DNA sequencing. DNA sequencing, large scale plasmid preparation and transfection of mammalian cells are done using standard procedures, and the cell culture supernatant containing the secreted enzyme is collected. The IC50 of this mutant for Val-boroPro inhibitor with 0.1 mM Ala-Pro-AFC substrate is measured at 12 nM compared to 20 nM for parent FAP plasmid and <0.5 nM for recombinant soluble DPPIV produced from plasmid #135. FIG. 14 shows IC50 determination on soluble secreted hFAP alpha dimer enzyme with mutations Q732E and N733D (Plasmid #94) compared to "wild-type" FAP alpha dimer enzyme (#122) for val-boroPro and val-nitriloPro inhibitors. Both proteins include a T229M mutation relative to published hFAP. Curve 1and 2 are #94 and #122 with val-boroPro respectively; curve 3 and 4 are #94 and #122 with val-nitriloPro respectively. The results show that this double mutation lowers the IC50 of both inhibitors Val-boroPro and Val-nitriloPro.

Example 4.6: Recombinant hFAP with mutations A347V, G349R, F351R and V352P, Q732E and N733D:

Plasmids #94 and #219 from above examples are spliced together to combine the sets of mutations therein. The 3' end of the gene is excised from plasmid #94 with EcoRV and NotI and ligated to #219 cut with the same enzymes to give a plasmid #254. DNA sequencing, large scale plasmid preparation and transfection of mammalian cells are done using standard procedures, and the cell culture supernatant containing the secreted enzyme is collected.

Example 4.7: Recombinant hFAP with mutations A347V, G349R, F351R and V352P and A657D:

Mutated segments from plasmid #219 and #233 are combined using the restriction enzymes EcoRV and NotI to excise the A657D mutation from #233 for insertion into #219. The resultant plasmid with the combined mutations is called #245.

Example 4.8: Recombinant chimeric hFAP-DPPIV with N679-N733 replaced by the corresponding human DPPIV residues N685–D739:

A unique EcoRI site overlaps the codons for amino acids 678-680 of human FAP. The region between this site and the histidine of the catalytic triad is replaced by the corresponding region of hDPPIV based on alignments of the sequences. This is accomplished in 2 stages. First the 3' end of hFAP from the EcoRI site to the 3' end is replaced by the corresponding region of DPPIV, then the C-terminal 24 amino acids of FAP are restored by overlap extension PCR using KOD thermostable polymerase (Novagen, Madison, WI, USA). The first step is accomplished by inserting a EcoRI – NotI PCR fragment made from a forward DPPIV primer (FAP-DPP4-RI-F: 5' ggatgataat cttgagcac tataaGAATT Caacagtcat gagcagagct 3', SEQ ID NO: 47) that inserts a EcoRI site at amino acid 684-686 of DPPIV and a reverse primer that places a Not I site immediately 3' of the DPPIV stop codon. After digestion with EcoRI and NotI, an approximately 250 nt fragment is gel purified and used to replace the EcoRI-Not I 3' fragment of sr hFAP/mouse FAP chimera in plasmid #23. This new plasmid #102, has no measurable enzymatic activity in supernatants of transfected mammalian cell line 293T. Restoration of activity is accomplished by restoring the 3' 27

codons from hFAP C-terminus and the human N-terminal 77 amino acids, leaving an internal portion of 55 amino acids from DPPIV.

The intended restoration is produced using overlap extension PCR. For Round 1 PCR Tube A, the template is plasmid #102 with the 5' PCR primer (FAP-DPP4-RI-F: 5' ggatgataat cttgagcac tataaGAATT Caacagtcat gagcagagct 3', SEQ ID NO: 47) paired with the mutagenic 3' reverse primer (DPP-FAP-R: 5' agg ccg gat aag ccA TGG TCT TCA TCA GTA TAC CAC ATT GCC TGG A 3', SEQ ID NO: 48). The latter has FAP homology at its 5' end and DPPIV homology at its 3' end, joining DPPIV D739 in-phase with hFAP H734. Tube B PCR, with hFAP as template, has the mutagenic (DPP-FAP-F 5' CAATGTGGTA TACTGATGAA GACCATggct tatccggcct gtccac 3', SEQ ID NO: 49) primer in the forward sense. This primer overlaps DPP-FAP-R. The reverse primer is hFAP-Not-R (5'ggt cgc tca gcg gcc gct tagtc tga caa aga gaa aca ctg ctt tag 3', SEQ ID NO: 35). The products of Round 1 PCR are mixed (0.5 µl each in a 50 µl reaction) and Round 2 PCR done with just the outermost primers FAP-DPP4-RI-F and hFAP-Not-R. The resulting PCR product of ca 260 nt is purified and cut with EcoRI and Not I, run on agarose and the band excised and DNA gel purified. This is then ligated to sr hFAP plasmid #122 from which the corresponding fragment had been excised. The resultant plasmid is #155. DNA sequencing, large scale plasmid preparation and transfection of mammalian cells are done using standard procedures, and the cell culture supernatant containing the secreted enzyme is collected which yields active enzyme.

Example 4.9: Recombinant chimeric hFAP-DPPIV with mutations A347V, G349R, F351R and V352P in the FAP portion and N679-N733 replaced by the corresponding human DPPIV residues:

The mutation in plasmids #155 and #219 are combined by inserting the EcoR V-Not I restriction fragment from #155 into the corresponding location in plasmid #219 to give plasmid #251. DNA sequencing, large scale plasmid preparation and transfection of mammalian cells are done using standard procedures, and the cell culture supernatant containing the secreted enzyme is collected. FIG. 15 shows Eadie-Hoftsee plots for determination of Km for FAP-DPPIV chimera produced from plasmid #155 in tissue culture supernatant compared to control FAP (#122). The gradient is the negative value of the Km and is 417 and 561 microMolar for plasmid #155 and #122 respectively. Plasmid #122 serves as wild-type control, and the chimera has a lower Km.

Example 4.10: Recombinant soluble hDPPIV with D663A mutation:

The intended mutations are produced using overlap extension PCR. The two overlapping mutagenic primers for this mutation are DPP4-A663-F (5' TCC CGG TGG GAG TAC TAT GCC TCA GTG TAC ACA GA 3', SEQ ID NO: 50), and DPP4-A663-R (5' TCT GTG TAC ACT GAG GCA TAG TAC TCC CAC CGG GA, SEQ ID NO: 51). The flanking non-mutagenic primers are DPPIV 1300-F (5' AAGACTGCAC ATTTATTACA AAAGGCACC 3', SEQ ID NO: 52) and DPP4-R (5' gtcggagcgg ccgcctaagg taaagagaaa cattgtttta tgaagtg 3', SEQ ID NO: 31). In Round 1, the template is plasmid #135 which contains DPPIV deleted for amino acids 1-38, fused to the immunoglobulin kappa chain secretory sequence in pSecTag2-B via an engineered Sfi I restriction site that leaves 6 vector-encoded amino acids at the N-terminus of the soluble protein (see Example above). In Round 2 the flanking primers generate an approx. 1.15 kb piece from the two Round 1 products. The PCR fragment and plasmid #135 are cut with BstX I and the internal approximately 625 nt fragment is replaced with the mutated fragment by ligating approximately 40 ng vector with 10 ng insert in a 5 microlitre ligation. The resultant plasmid after transformation and screening is #266. This plasmid is transfected into 293T fibroblast cells and secreted enzyme collected in the culture supernatant.

Mutation of this residue in native, membrane-bound mouse DPPIV to alanine or glycine has been reported, however the mutation was characterized as not significantly modifying the expression or enzymatic properties of the resultant enzyme. (David, F. et al. 1993. J. Biol. Chem., 268, 17247-17252.). Therefore, the corresponding human DPPIV D663 (mouse D657) aspartate residue would be expected to be an unlikely candidate for altering enzymatic properties. However, the secreted form of human DPPIV D633A mutant described in this Example has novel properties that are significantly different from its parent.

The effect of the D663 mutation on selected properties of soluble DPPIV are shown in FIG. 13. FIG. 13A shows that the pH-activity profile is less sensitive to pH, compared to the control plasmid #135. The latter (plasmid #135) shows the typical DPPIV pH profile reported in the literature. FIG. 13B shows measurement of IC50 for Val-boroPro inhibitor in assays in which substrate and inhibitor were added simultaneously. A significant change in IC50 in #266 (arrow 1) compared to wild-type soluble DPPIV (arrow 2) is seen. Arrow 3 shows wild-type FAP for comparison. The DPPIV IC50 is abnormally high because pre-incubation with

inhibitor was deliberately omitted, so that alterations in slow-binding kinetics would be apparent. FIG. 13C shows that the response to inhibition by val-boroPro is altered in the D663A mutant to be more sensitive when inhibitor and substrate are added simultaneously as demonstrated by the greater difference in the slope of the curves for the wild-type depending on how long a pre-incubation was allowed before substrate addition. i.e. the slow-binding kinetics is diminished at least when inhibitor is added 30 sec before substrate, thereby becoming more similar to wild-type FAP. FIG. 13D shows activity versus Ala-Pro-AFC substrate concentration for Km determination for plasmid #135 (control) and D663A mutant, plasmid #266. Km values of 51 microMolar for mutant #266 and 14 microMolar for wild-type DPPIV (the latter indistinguishable from published values) were found. Thus, the Km for Ala-Pro-AFC substrate is altered. Second, the slow-binding property for proline-2-boronic acid inhibitors, typified by its response to Val-boroPro, is diminished if not eliminated. Furthermore, its pH profile shows a marked resemblance to that of FAP rather than DPPIV.

Example 4.11: Measurement of inhibition of mutant FAP dimer enzyme from plasmids #217, 219, 251, 255, 257, 233 and 245 by Val-nitriloPro compared to control FAP (#122) and DPPIV (#135):

Soluble recombinant enzyme was produced in the supernatant medium from transfected HEK 293T cells. Assays were conducted as in Example 1.7 and contained 0.2mM Ala-Pro-AFC substrate. Production of fluorescence was monitored continuously for 20 min, and linear rates were extracted from the data. FIG. 11 shows the effect of FAP mutations described in the examples above on the percent inhibition by the inhibitor val-nitriloPro (valine-2-nitrilo-pyrollidine) at a range of concentrations. Soluble FAP from plasmid #217 shows little effect on inhibition compared to #122 which is used as a control. In contrast, all mutants containing the hFAP A347V, G349R, F351R and V352P mutations (plasmids 219, 251, 255, 257) show increased sensitivity to this inhibitor, in the range of 60-70% inhibition at 4 microMolar. The only exception is plasmid #245 which carries the additional A657D mutation. The latter (plasmid #245) and the A657D mutant #233, produce FAP alpha dimer enzymes with an even greater susceptibility more similar to DPPIV (e.g., approximately 90% at 4 microMolar).

The responses of these FAP alpha dimer mutants at 4 microM Val-nitriloPro are summarized in Table 2, and can be roughly divided into 3 classes based on degree of inhibition. Less than 50% inhibition: wild type FAP and FAP mutant #217 (Y124H, A207S). 60-70% inhibition: FAP mutants containing the combined A347V, G349R, F351R, V352P set of mutations found in plasmid #219 and derivatives thereof #251, #254, and #257. Greater than 90% inhibition: wild type DPPIV (#135), and FAP mutants #233, #245 which both contain the A657D mutation.

Table 2. % inhibition of FAP mutants by val-nitriloPro at 4 micromolar

Plasmid	#217	#219	#233	#245	#251	#255	#257	#122 hFAP	#135 hDPP IV
% inhibition	45	65	95	95	65	70	68	43	100

Since plasmids #245 and #233 both share the A657D mutation, it thus inferred that this single amino acid change can significantly alter this particular property of FAP to resemble DPPIV. Mutation of this FAP residue to aspartate is a preferred embodiment, as is its mutation to any other amino acid but in particular to amino acids with less bulky side chains including glycine, serine, cysteine, and valine.

Example 4.12: Recombinant soluble hDPPIV with V354A, R356G, R358F, P359V mutations:

The "R356" region of DPPIV constitutes a loop, the apex of which, based on crystal structure analysis, comprises the R357, F357 and R358 residues which are exposed in the interior of the active site. Four residues in this region are changed to the corresponding FAP residues using overlap extension PCR to make the loop more hydrophobic overall. The intended mutations are produced using overlap extension PCR. For Round 1 PCR, Tube A, the PCR primers are SwaI-F (5' GACATTTATGATTTAAATAAAAGGCAGCTGATTAC AGAAGAG 3', SEQ ID NO: 53) and R356-R: 5' CTG AAG CGA AAA AAC CTC CAG CCC AGC CAG TAG TAC TCA TTC AAT G 3' (SEQ ID NO: 54) and Tube B contains primers R356-F: 5' GCTGGAGGTT TTTTCGCTTC AGAACCTCAT TTTACCCTTG ATGGT 3' (SEQ ID NO: 55) and DPPIV_BspEI-R sequencing primer (5' TAG TAC TGA CAC CTT TCC GGA TTC AGC TCA 3', SEQ ID NO: 56) with cloned DPPIV cDNA plasmid #135 as template. The products of the first reactions are combined with the external

primers SwaI-F and DPPIV_BspEI-R in Round 2, and the resulting product cut with SwaI and BspEI giving an approx 940 nt fragment and ligated into sr hDPPIV plasmid #135 prepared with same enzymes. Cycling parameters for both rounds are: initial denaturation at 94°C for 40 sec; then 25 cycles of 94°C for 15 sec, 54°C for 15 sec and 72°C for 1 min. After cycling, extension is continued at 72°C for 5 min followed by cooling to 4°C. PCR products are isolated using a commercial kit (QIAquick PCR purification Kit, Qiagen), cut with Swa I and BspE I restriction enzymes, run on an agarose gel, and the approximately 600 nt fragment isolated using a commercially available kit (QIAquick Gel extraction Kit, Qiagen). Expression of soluble enzyme follows the protocol in the preceding examples for soluble recombinant proteins.

Example 4.13: Recombinant soluble hDPPIV with H126Y mutation:

The H126Y mutation can be introduced in a single round of PCR due to proximity to a unique Swa I restriction site. A single reverse primer (H124Y-R: 5' GCCTTTTATTTAAAT CAT AAA TGT CAT ATG AAG CTG TGT AGG AAT aCC TCC ATT 3', SEQ ID NO: 57; Swa I site underlined), is coupled with the Sfi-DPP4 primer (5' GTAGTCGGCC CAGCCGGCC AGTCGCAAAA CTTACACTCT AACTGATTAC TTAAAAAAT 3', SEQ ID NO: 30) to generate a PCR fragment from DPPIV template (e.g. plasmid #135) that is digested with Sfi I-Swa I restriction enzymes and used to replace the corresponding fragment at the 5' end of the hDPPIV gene in plasmid #135 using standard ligation techniques.

Example 4.14: Recombinant soluble hDPPIV with S209A mutations:

The intended mutation is produced using overlap extension PCR. For Round 1 PCR, Tube A, the PCR primers are SwaI-F (5' GACATTTATG ATTTAAATAA AAGGCAGCTG ATTACAGAAG AG 3', SEQ ID NO: 53) and S209A-R (5' ACC ACC ACA GAG CAG CGT AGG CAC TGA AGA CT 3', SEQ ID NO: 58) and Tube B contains primers S209A-F (5' AGTCTTCAGT GCCTACTaTG CTCTGTGGTG GT 3', SEQ ID NO: 59) and DPPIV_BspEI-R sequencing primer (5' TAG TAC TGA CAC CTT TCC GGA TTC AGC TCA 3', SEQ ID NO: 56) with cloned DPPIV cDNA in plasmid #135 as template. The products of the first reactions are combined with the external primers SwaI-F and DPPIV_BspEI-R in Round 2, and the resulting product cut with SwaI and BspEI giving an

approx 940 nt fragment and ligated into sr hDPPIV plasmid #135 prepared with the same enzymes.

Example 5.1: Soluble recombinant human-mouse FAP chimera with N-terminal 77 amino acids (excludes vector-derived residues) from mouse FAP:

The Sfi I-XbaI fragment of plasmid #13 containing the entire coding region of human FAP, but with a single T229M amino acid change, is replaced by the corresponding fragment from mouse FAP, generated by PCR. The primer Sfi-FAP-B (5' GTAGTCGGCC CAGCCGGCCA CAAAGAGAGC TCTTACCCTG AAGGATATTT TAAATG 3', SEQ ID NO: 13) and a reverse primer mFAP45 (5' TTC CAT TGG GCC CAC GTG GTG 3', SEQ ID NO: 60) located 3' of the conserved Xba I site (the latter overlaps amino acids 113-115), were used to amplify the 5' end of the mouse gene between amino acids 38 –115. The PCR product is digested with Sfi I and Xba I restriction enzymes and inserted into plasmid #13 from which the 5' end of the gene is excised with the same enzymes. The resultant plasmid fuses the vector-encoded immunoglobulin secretion sequence to codon #39 of the mouse FAP which is, in turn fused to the human FAP at amino acid 114. The resulting plasmid, called #23, contains 77 amino acids of mouse FAP and 683 amino acids of human FAP. Because of homology between the two species, there are 13 amino acid differences in the chimeric segment compared to the wholly human FAP alpha dimer enzyme.

Example 5.2: Soluble recombinant human-mouse FAP chimera with N-terminal 77 amino acids only (excludes vector-derived residues) from human FAP:

A plasmid pcDNA FAP#5 containing mouse FAP cDNA corresponding to the published sequence (GenBank Accession number Y10007) is obtained from a commercial source cloned into the poly linker of a commercially available vector pcDNA (InVitrogen). This cDNA is excised using EcoRI and NotI restriction sites in the flanking polylinker and ligated to pSecTag2-B secretion vector (InVitrogen), giving plasmid #18.

A truncated approximately 260 nt 5' fragment of human FAP deleting the first 38 amino acids of hFAP and inserting a Sfi I restriction site adjacent to the Thr39 codon is derived by PCR. This allows an in-phase junction to the vector Ig-kappa secretion sequence at the Sfi I site. The 5' PCR primer (Sfi-FAP-B 5' GTAGTCGGCC CAGCCGGCCA CAAAGAGAGC TCTTACCCTG AAGGATATTT TAAATG 3', SEQ ID NO: 13 (Sfi I site

underlined) makes residue #39 a lysine, as found in mouse FAP. PCR of FAP cDNA with this primer and a reverse primer (FAP porbe 5' tgaaataataGtcacttgaggctatcatt 3') located 3' of the common, conserved unique Xba I site, gives a PCR product of approximately 700 nt. A Sfi I-Xba I double digest on plasmid #18 is used to remove the native 5' end of mouse FAP up to the internal Xba I site at codon 114. and to cut the FAP PCR product. The appropriate fragments of >5 kb and approximately 259 nt respectively are isolated from an agarose gel, ligated, and transformants screened. The resulting plasmid, called #29, contains 77 amino acids of human FAP and 683 amino acids of mouse FAP. Because of homology between the two species, there are a total of 13 amino acid differences in the chimeric segment compared to the wholly mouse FAP.

The N-terminus of the final mature a.a. sequence of cleaved secreted product will contain 6 a.a from the vector, DAAQPA (SEQ ID NO: 14), fused to the truncated FAP sequence, of which the first 13 amino acids are TKRALTLKDILNG (SEQ ID NO: 15). FIG. 9 shows soluble secreted FAP alpha dimer activity in tissue culture supernatant from plasmids #23, #29 and #43 measured by production of fluorescence from Ala-Pro-AFC substrate at pH 8.1.

Example 5.3: Production of soluble secreted mouse FAP:

The approximately 250 nt SfiI-XbaI 5' fragment of plasmid #23 is ligated to similarly-cut plasmid #29 to generate a plasmid #43 for production of soluble mouse FAP amino acids 39-760 of which the first 13 amino acids are TKRALTLKDILNG (SEQ ID NO: 15). The N-terminus of the cleaved mature secreted mouse FAP will contain 6 amino acid from the vector (i.e., DAAQPA, SEQ ID NO: 14). This plasmid produces soluble secreted recombinant FAP dimer activity when expressed in HEK 293T cells, as shown in FIGs. 5 and 9.

Example 5.4: Soluble human FAP chimera with amino acids #269-557 substituted with the corresponding mouse FAP residues:

The ClaI – EcoRV region of sr hFAP in plasmid #122 is replaced with a PCR fragment of mouse FAP containing the corrresponding region. The PCR primers are designed to introduce the ClaII – EcoRV sites into the mouse fragment to facilitate cloning. The

resulting plasmid, #279, gives low but measurable FAP activity. The residues in murine wild type FAP alpha dimer enzyme correspond to those in the human homolog.

Example 5.5: Soluble human FAP chimera with amino acids # 269-448 substituted with the corresponding mouse FAP residues:

The region noted corresponds to the Cla I – Ban I region of sr hFAP in plasmid #122, which is replaced with a PCR fragment of wild type mouse FAP alpha dimer enzyme containing the corresponding amino acids. Since mFAP does not have a ClaI restriction site, PCR primers were designed to a chimeric ClaI-EcoRV fragment (FAP residues introduce the Cla I site into the mouse fragment to facilitate cloning. The resulting plasmid, #286, gives good FAP enzymatic activity. The residues in murine wild type FAP alpha dimer enzyme correspond to those in the human homolog.

Example 5.6: Soluble human FAP chimera with mouse FAP amino acids # 449-557:

This hFAP-mouse chimera corresponds to replacement of the BanI – EcoRV fragment of hFAP (amino acids # 449-557) with the mouse equivalent is made by creating a chimeric ClaI-EcoRV fragment by PCR. This is accomplished by digesting plasmid #122 (sr hFAP) with ClaI and EcoRV, isolating the approximately 0.9 kb fragment, mixing it with plasmid #264 (sr hFAP with ClaI-EcoRV from mouse) and jointly cutting with BanI. DNA is purified from the digest with a kit (QIAquick PCR purification, Qiagen) and self ligated. Chimeric molecules ligated at the BanI site are selected by PCR with a 5' human primer hFAP-Cla-F and a mouse reverse primer mFAP-RV-R. The PCR product is digested with ClaI and EcoRV and ligated to similarly-cut plasmid #122. This gives plasmid #279. A map of this plasmid is shown in FIG. 16A and its activity is shown in FIGs. 16B and 16C relative to other mouse-human chimeras.

Example 5.7: Soluble human FAP chimera with mouse FAP amino acids # 449-557 with reversion of mouse region K512 – F518 to the human residues:

The hFAP-mouse BanI – EcoRV chimera (plasmid #279) is altered to restore the human residues E512 – L518 using overlap-extension PCR with #279 as template. The overlapping forward and reverse mutagenic PCR primers were designed to introduce the desired changes into separate overlapping halves of the mouse fragment which is then joined

in the second round of PCR, cut with ClaI and EcoRV. The resulting plasmid, #326, gave improved FAP activity over #279, indicating that changes in these 7 residues in human FAP were not well tolerated, at least in the context of a human-mouse chimera. A map of representative human-mouse chimeras of soluble FAP is shown in FIG. 16A. These show the relevant mouse and human portions of the chimeras. In the case of #319 and #326, the location of additional point mutations is shown. All are derived from plasmid #122 and so also carry the T229M change. The relative activity of tissue culture supernatants of representative hu-mouse FAP chimeras Cla I-EcoRV interval are shown in FIG. 16B and 16C. The reversion of mouse region K512 – F518 to the human residues in #326 has a dramatic effect on resultant activity. In all cases, the same amount of plasmid was used to transfect HEK 293T cells and the an aliquot of the supernatants assayed with Ala-Pro-AFC.

Example 6.1: Soluble forms treated with Val-boroPro or other proline-2-boronic acid that slowly regain activity ("Slow release"):

Soluble recombinant DPPIV or its D663A mutant in a pharmaceutically acceptable injectable preparation is treated with Val-boroPro at a concentration of approximately 0.5 nM or higher but typically not greater than 10 micromolar, for a period of approximately 1 -15 min or longer, during which time strong inhibitory binding of the inhibitor occurs to the wild-type enzyme or relative strong binding in the case of the D663A mutant.

Example 6.2: Inhibitor-bound recombinant soluble FAP A657D mutant with slow-release of activity:

Soluble recombinant FAP A657D mutant in a pharmaceutically acceptable injectable preparation is treated with Val-boroPro at a concentration of approximately 0.5 nM or higher but typically not greater than 10 micromolar, for a period of approximately 1 -15 min or longer, during which time a complex of intermediate duration forms.

References

1. Dinarello, C.A. Interleukin-1 and interleukin-1 antagonism. *Blood* 77, 1627-1652 (1991).
2. Dinarello, C.A. & Wolff, S.M. The role of interleukin-1 in disease. *N. Engl. J. Med.* **328**, 106-113 (1993).

3. Schwab, J.H., Anderle, S.K., Brown, R.R., Dalldorf, F.G. & Thompson, R.C. Pro- and anti-inflammatory roles of interleukin-1 in recurrence of bacterial cell wall-induced arthritis in rats. *Infect. Immun.* 59(1991).

4. Feige, U., Karbowoski, A., Rordorf-Adam, C. & Pataki, A. Arthritis induced by continuous infusion of hr-interleukin-1α into the rabbit knee joint. *Int. J. Tissue React.* **11**, 225-238 (1989).

5. Mandrup-Poulsen, T., Helqvist, S., Molvig, J., Wogensen, L.D. & Nerup, J. Cytokines as immune effector molecules in autoimmune endocrine diseases with special reference to insulin-dependent diabetes mellitus. *Autoimmunity* **4**, 191-218 (1989).

6. Fischer, E., Marano, M.A., Bareber, A.E. & al, e. Comparison between effects of interleukin-1a administration and sublethal endotoxemia in primates. *Am. J. Physiol.* **261**, R442-R452 (1991).

7. Bernard, C. & Tedgui, A. Cytokine network and the vessel wall. Insights into septic shock pathogenesis. *Eur. Cytokine Net.* **3**, 19-33 (1992).

8. Opp, M.R. & Krueger, J.M. Interleukin 1-receptor antagonist blocks interleukin-1 induced sleep and fever. *Am. J. Physiol.* **260**, R453-R457 (1991).

9. Rodriguez, R.M., Corwin, H.L., Gettinger, A. & al, e. Nutritional deficiencies and blunted erythropoietin response as causes of the anemia of critical illness. *J. Crit. Care* **16**, 36-41 (2001).

10. Vincent, J.L., Baron, J.F., Gattinoni, L. & al, e. Anemia and blood transfusions in the critically ill: An epidemiological observational study. *JAMA* **288**, 1499-1507 (2002).

11. Krafte-Jacobs, B., Levetown, M.L., Bray, G.L. & al, e. Erythropoietin response to critical illness. *Crit. Care Med.* **22**, 821-826 (1994).

12. Frede, S., Fandrey, J., Pagel, H., Hellwig, T. & Jelkmann, W. Erythropoietin gene expression is suppressed after lipopolysaccharide or interleukin-1 beta injections in rats. *Am. J. Physiol.* **273**, R1067-R1071 (1998).

13. Jelkmann, W. Proinflammatory cytokines lowering erythropoietin production. *J. Interferon Cytokine Res.* **18**, 555-559 (1998).

14. Rambaldi, A., Torcia, M., Bettoni, S. & al, e. Modulation of cell proliferation and cytokine production in acute myeloblastic leukemia by interleukin-1 receptor antagonist and lack of its expression by leukemia cells. *Blood* **78**, 3248-3253 (1991).

15. Griffin, J.D. et al. Secretion of interleukin-1 by acute myeloblastic leukemia cells in vitro induces endothelial cells to secrete colony stimulating factors. *Blood* **70**, 1218-1221 (1987).

16. Bagby, G.C.J., Dinarello, C.A., Neerhout, R.C., Ridgway, D. & McCall, E. Interleukin 1-dependent paracrine granulopoiesis in chronic granulocytic leukemia of the juvenile type. *J. Clin. Invest.* **82**, 1430-1436 (1988).

17. Bodel, P. & Atkins, E. Release of endogenous pyrogen by human monocytes. *N. Engl. J. Med.* **276**, 1002-1008 (1967).

18. Cozzolino, F. et al. Interleukin 1 as an autocrine growth factor for acute myeloid leukemia cells. *Proc. Natl. Acad. Sci. USA* **86**, 2369-2373 (1989).

19. Sakai, K. et al. Autocrine stimulation of interleukin 1β in acute myelogenous leukemia cells. *J. Exp. Med.* **166**, 1597-1602 (1987).

20. Estrov, Z., Kurzrock, R., Wetzler, M. & al, e. Suppression of chronic myelogenous leukemia colony growth by interleukin-1 (IL-1) receptor antagonist and soluble IL-1 receptors: a novel application for inhibitors of IL-1 activity. *Blood* **78**, 1476-1484 (1991).

21. Peled, T. et al. Effect of M20 interleukin-1 inhibitor on normal and leukemia human myeloid progenitors. *Blood* **79**, 1172-1177 (1992).
22. Akira, S., Taga, T. & Kishimoto, T. Interleukin-6 in biology and medicine. *Adv. Immunol* **54**, 1-78 (1993).
23. Suematsu, S. et al. IgG1 plasmacytosis in interleukin 6 transgenic mice. *Proc. Natl. Acad. Sci. USA* **86**, 7547-7551 (1989).
24. Suematsu, S. et al. Generation of transplantable plasmacytomas with t(12:15) in IL-6 transgenic mice. *Proc. Natl. Acad. Sci. USA* **89**, 232-235 (1992).
25. Dejana, E. et al. Interleukin 1 promotes tumor cell adhesion to cultured human endothelial cells. *J. Clin. Invest.* **82**, 1466-1470 (1988).
26. Giavazzi, R. et al. Interleukin 1-induced augmentation of experimental metastases from a human melanoma in nude mice. *Cancer Res.* **50**, 4771-4775 (1991).
27. Vidal-Vanaclocha, F., Amezaga, C., Asumendi, A., Kaplanski, G. & Dinarello, C.A. Interleukin-1 receptor blockade reduces the number and size of murine B 16 melanoma hepatic metastases. *Cancer Res.* **54**, 2667-2672 (1994).
28. Morimoto, C. & Schlossman, S.F. The structure and function of CD26 in the T-cell immune response. *Immunological Reviews* **161**, 55-70 (1998).
29. von Bonin, A., Huhn, J. & Fleischer, B. Dipeptidyl-peptidase IV/CD26 on T cells: analysis of an alternative T-cell activation pathway. *Immunol. Rev.* **161**, 55-70 (1998).
30. Niedermeyer, J. et al. Mouse fibroblast-activation protein Conserved *Fap* gene organization and biochemical function as a serine protease. *Eur. J. Biochem.* **254**, 650-654 (1998).
31. Scanlan, M.J. et al. Molecular cloning of fibroblast activation protein, a member of the serine protease family selectively expressed in stromal fibroblasts of epithelial cancers. *Proc. Natl. Acad. Sci. USA* **91**, 5657-5661 (1994).
32. De Meester, I., Korom, S., Van Damme, J. & Scharpe, S. CD26, let it cut or cut it down. *Immunol. Today* **20**, 367-375 (1999).
33. Marguet, D. et al. Enhanced insulin secretion and improved glucose tolerance in mice lacking CD26. *Proc. Natl. Acad. Sci. USA* **97**, 6874-6879 (2000).
34. Mentlein, R., Gallwitz, B. & Schmidt, W.E. Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7-36)amide, peptide histidine methionine and is responsible for their degradation in human serum. *Eur. J. Biochem.* **214**, 829-835 (1993).
35. Park, J.E. et al. Fibroblast activation protein, a dual specificity serine protease expressed in reactive human tumor stromal fibroblasts. *J. Biol. Chem.* **274**, 36505-36512 (1999).
36. Garin-Chesa, P., Old, L.J. & Rettig, W.J. Cell surface glycoprotein of reactive stromal fibroblasts as a potential antibody target in human epithelial cells. *Proc. Natl. Acad. Sci. USA* **87**, 7235-7239 (1990).
37. von Bonin, A., Huhn, J. & Fleischer, B. Dipeptidyl-peptidase IV/CD26 on T cells: analysis of an alternative T-cell activation pathway. *Immunological Reviews* **161**, 43-53 (1998).
38. Yaron, A. & Naider, F. Proline-dependent structural and biological properties of peptides and proteins. *Crit. Rev. Biochem. Mol. Biol.* **28**, 31-81 (1993).
39. Demuth, H.-U. & Heins, J. Catalytic mechanism of dipeptidyl peptidase IV. in *Dipeptidyl peptidase IV (CD26) in metabolism and immune response* (ed. Fleischer, B.) 1-34 (R.G. Landes Co., 1995).

40. Coutts, S.J. et al. Structure-activity relationships of boronic acid inhibitors of dipeptidyl peptidase IV. 1. Variation of the P_2 position of X_{aa}-boroPro dipeptides. *J. Med. Chem.* **39**, 2087-2094 (1996).

41. Tsuji, E. et al. An active site mutation (Gly^{633} to Arg) of dipeptidyl peptidase IV causes its retention and rapid degradation in the endoplasmic reticulum. *Biochemistry* **31**, 11921-11927 (1992).

42. Dinarello, C.A. The biological properties of interleukin-1. *Eur. Cytokine Net.* **5**, 517-531 (1994).

Equivalents

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by examples provided, since the examples are intended as a single illustration of one aspect of the invention and other functionally equivalent embodiments are within the scope of the invention. Various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims. The advantages and objects of the invention are not necessarily encompassed by each embodiment of the invention.

All references, patents and patent applications that are recited in this application are incorporated by reference herein in their entirety.

What is claimed is:

Claims

1. A method for down-regulating an immune response comprising administering to a subject in need thereof a FAP alpha dimer enzyme in an amount effective to down-regulate an immune response.

2. The method of claim 1, wherein the immune response is an IL-1 mediated condition.

3. The method of claim 1, wherein the immune response is an abnormal immune response.

4. The method of claim 3, wherein the abnormal immune response is selected from the group consisting of inflammation, autoimmune disease, sepsis, graft versus host disease, transplant rejection, toxic shock syndrome, allergy, asthma, atherosclerosis, osteoarthritis, and Guillain-Barre's syndrome.

5. The method of claim 3, wherein the abnormal immune response is subsequent to an infection.

6. The method of claim 5, wherein the infection is an RSV infection.

7. The method of claim 4, wherein the autoimmune disease is selected from the group consisting of rheumatoid arthritis, insulin dependent diabetes (type I diabetes), inflammatory bowel disease, autoimmune thyroiditis, systemic lupus erythematosus (SLE), uveitis, hemolytic anemias, rheumatic fever, Crohn's disease, Guillain-Barre's syndrome, psoriasis, Graves' disease, myasthenia gravis, glomerulonephritis, autoimmune hepatitis and multiple sclerosis.

8. The method of claim 1,wherein the subject does not have cancer or the subject does not have a predisposition to cancer.

9. The method of claim 1, further comprising administering to the subject a second agent.

10. The method of claim 9, wherein the second agent is an anti-inflammatory agent.

11. The method of claim 9, wherein the second agent is an immunosuppressant.

12. The method of claim 9, wherein the second agent is an anti-infective agent.

13. The method of claim 12, wherein the anti-infective agent is an anti-bacterial agent.

14. The method of claim 12, wherein the anti-infective agent is an anti-viral agent.

15. The method of claim 12, wherein the anti-infective agent is an anti-fungal agent.

16. The method of claim 12, wherein the anti-infective agent is an anti-parasitic agent.

17. The method of claim 12, wherein the anti-infective agent is an anti-mycobacterial agent.

18. The method of claim 1, wherein the FAP alpha dimer enzyme is wild type FAP alpha dimer enzyme.

19. The method of claim 1, wherein the FAP alpha dimer enzyme is a truncation mutant.

20. The method of claim 1, wherein the FAP alpha dimer enzyme is a fusion or chimeric protein.

21. The method of claim 20, wherein the fusion or chimeric protein comprises a sequence selected from the group consisting of a secretion sequence, a purification sequence, an epitope, a linker, a protein degradation sequence, a protease cleavage site, a self-cleaving affinity tag, a tissue localization sequence and a peptide or protein ligand.

22. The method of claim 21, wherein the secretion sequence is a G-CSF leader sequence or an Ig-kappa leader sequence.

23. The method of claim 21, wherein the purification sequence is selected from the group consisting of a GST sequence tag, a hexahistidine or polyhistidine tag, a Protein A tag, a biotin tag, a chitin tag, and a maltose binding domain.

24. The method of claim 21, wherein the epitope is selected from the group consisting of a hemaglutinnin tag, a FLAG tag, a V5 tag, a myc tag and a T7 tag.

25. The method of claim 21, wherein the protein degradation sequence is a PEST sequence.

26. The method of claim 21, wherein the protease cleavage site is selected from the group consisting of enterokinase, factor Xa protease, thrombin, TEV protease, PreScission protease, Furin, and Genenase.

27. The method of claim 20, wherein the fusion or chimeric protein comprises an amino acid substitution of Q732E or N733D.

28. The method of claim 1, wherein the FAP alpha dimer enzyme is a heterodimer.

29. The method of claim 28, wherein the heterodimer is a heterodimer of a FAP alpha monomer and a DPPIV/CD26 monomer.

30. The method of claim 1, wherein the FAP alpha dimer enzyme comprises an amino acid substitution relative to wild type FAP alpha dimer.

31. The method of claim 30, wherein the amino acid substitution is present in the β-propeller domain.

32. The method of claim 30, wherein the amino acid substitution is selected from the group consisting of Y124, A207, A347, G349, F351, V352.

33. The method of claim 30, wherein the amino acid substitution is selected from the group consisting of Y124H, A207S, A347V, G349R, F351R, V352P.

34. The method of claim 30, wherein the amino acid substitution is present in the catalytic domain.

35. The method of claim 34, wherein the amino acid substitution is in amino acid A657.

36. The method of claim 30, wherein the amino acid substitution is A657D.

37. The method of claim 30, wherein the amino acid substitution is Y124H or A207S.

38. The method of claim 30, wherein the amino acid substitution is A347V, G349R, F351R or V352P.

39. The method of claim 30, wherein the amino acid substitution is present in the entrance to the catalytic site.

40. The method of claim 39, wherein the entrance to the catalytic site is an apical entrance.

41. The method of claim 40, wherein the amino acid substitution is selected from the group consisting of G64D, Q65H, V299A, D301Q, T354E, V356H, S358T, Y359L, F401E, R402A, V403L, Q405S, T452S, A453V, D457K and Y458E.

42. The method of claim 39, wherein the entrance is a side entrance.

43. The method of claim 42, wherein the amino acid substitution is selected from the group consisting of N49K, G50N, F52Y, S53R, Y54L, T56L, F57Y, F58S, P59L, S71Q, D73E, S91E, R93S, M95F, K96D, S97E, V98F, N99G, A100H, S116Y, D117N, S119V, L121Q and Y124H.

44. The method of claim 30, wherein the amino acid substitution is present at an N-linked glycosylation site.

45. The method of claim 44, wherein the N-linked glycosylation site is selected from the group consisting of N49, N92, N99, N227, N314 and N679.

46. The method of claim 44, wherein the amino acid substitution is at T51, T94, S101, T229, S316 or T681.

47. The method of claim 44, wherein the amino acid substitution is at N227 and T229.

48. The method of claim 44, wherein the amino acid substitution is T229M.

49. The method of claims 1-47 or 48, wherein the FAP alpha dimer enzyme is soluble.

50. The method of claim 30, wherein the amino acid substitution alters disulfide bond formation.

51. The method of claim 50, wherein the amino acid substitution introduces a disulfide bond.

52. The method of claim 51, wherein the amino acid substitution is selected from the group consisting of H378C and A386C.

53. The method of claim 51, wherein the amino acid substitution is selected from the group consisting of L48C, N742C, M683C and I713C.

54. The method of claim 50, wherein the amino acid substitution removes a disulfide bond.

55. The method of claim 1, wherein the FAP alpha dimer enzyme is PEGylated.

56. The method of claim 55, wherein the FAP alpha dimer enzyme is PEGylated at a lysine.

57. The method of claim 55, wherein the FAP alpha dimer enzyme is PEGylated at a cysteine.

58. The method of claim 55, wherein the FAP alpha dimer enzyme is PEGylated at a cysteine introduced at position 95, 161, 173, 191, 219, 334, 372, 382, 436, 437, 445, 460, 486, 492, 499, 505, 509, 510, 521, 532, 533 564, 583, 591, 606, 616, 642, 670, 678, 715, 753, 91, 148, 263, 323, 343, or 444.

59. The method of claim 55, wherein the PEGylated FAP alpha dimer enzyme comprises a mutation in one or more amino acid positions selected from a group consisting of 95, 161, 173, 191, 219, 334, 372, 382, 436, 437, 445, 460, 486, 492, 499, 505, 509, 510, 521, 532, 533 564, 583, 591, 606, 616, 642, 670, 678, 715, 753, 91, 148, 263, 323, 343 and 444.

60. The method of claim 1, wherein the FAP alpha dimer enzyme is a dimerization domain mutant.

61. The method of claim 60, wherein the dimerization domain mutant lacks residues comprised of P232-I250 of wild type FAP alpha dimer enzyme and comprises residues P234-V254 of wild type DPPIV.

62. The method of claim 41, wherein the dimerization domain mutant lacks residues F706-D731 of wild type FAP alpha dimer enzyme or some portion thereof and comprises residues F713-D738 of wild type DPPIV or some portion thereof.

63. The method of claim 60, wherein the dimerization domain mutant comprises an amino acid substitution of T248C.

64. The method of claim 1, wherein the FAP alpha dimer enzyme lacks residues N679-N733 from wild type FAP alpha dimer enzyme and comprises residues N685-D739 of wild type DPPIV.

65. The method of claim 30, wherein the amino acid substitution is present in the cytoplasmic domain.

66. The method of claim 30, wherein the amino acid substitution is present in the transmembrane domain.

67. The method of claim 1, wherein the FAP alpha dimer enzyme lacks a cytoplasmic domain.

68. The method of claim 1, wherein the FAP alpha dimer enzyme lacks a transmembrane domain.

69. The method of claim 1, wherein the FAP alpha dimer enzyme lacks a cytoplasmic and transmembrane domain.

70. The method of claim 1, wherein the FAP alpha dimer enzyme lacks residues corresponding to 1-37 from wild type FAP alpha dimer enzyme.

71. The method of claim 1, wherein the FAP alpha dimer enzyme is soluble.

72. The method of claim 71, wherein the FAP alpha dimer enzyme comprises an amino acid substitution of T229M.

73. The method of claim 1, wherein the FAP alpha dimer enzyme comprises an amino acid sequence of SEQ ID NO: 4.

74. The method of claim 1, wherein the FAP alpha dimer enzyme is administered as a protein.

75. The method of claim 1, wherein the FAP alpha dimer enzyme is administered as a nucleic acid.

76. The method of claim 2, wherein the IL-1 is IL-1 alpha or IL-1 beta.

77. A composition comprising

a FAP alpha dimer enzyme in a pharmaceutically acceptable carrier, wherein the composition is sterile and lacks an adjuvant.

78. A composition comprising

a FAP alpha dimer enzyme in a pharmaceutically acceptable carrier, and

a non-adjuvant second agent.

79. The composition of claim 78, wherein the non-adjuvant second agent is an anti-inflammatory agent.

80. The composition of claim 78, wherein the non-adjuvant second agent is an immunosuppressant.

81. The composition of claim 78, wherein the preparation is sterile.

82. The composition of claim 77 or 78, wherein the FAP alpha dimer enzyme is wild type FAP alpha dimer enzyme.

83. The composition of claim 77 or 78, wherein the FAP alpha dimer enzyme is a truncation mutant.

84. The composition of claim 77 or 78, wherein the FAP alpha dimer enzyme is a fusion or chimeric protein.

85. The composition of claim 84, wherein the fusion or chimeric protein comprises a sequence selected from the group consisting of a secretion sequence, a purification sequence, an epitope, a linker, a protein degradation sequence, a protease cleavage site, a tissue localization sequence, a peptide or protein ligand.

86. The composition of claim 85, wherein the secretion sequence is a G-CSF leader sequence or an Ig-kappa leader sequence.

87. The composition of claim 85, wherein the purification sequence is selected from the group consisting of a GST sequence tag, a hexahistidine or polyhistidine tag, a Protein A tag, a biotin tag, a chitin tag, and a maltose binding domain.

88. The composition of claim 85, wherein the epitope is selected from the group consisting of a hemaglutinnin tag, a FLAG tag, a V5 tag, a myc tag and a T7 tag.

89. The composition of claim 85, wherein the protein degradation sequence is a PEST sequence.

90. The composition of claim 85, wherein the protease cleavage site is selected from the group recognized by enterokinase, factor Xa protease, thrombin, TEV protease, PreScission protease, Furin, Genenase.

91. The composition of claim 84, wherein the fusion or chimeric protein comprises an amino acid substitution of Q732E or N733D.

92. The composition of claim 77 or 78, wherein the FAP alpha dimer enzyme is a heterodimer.

93. The composition of claim 92, wherein the heterodimer is a heterodimer of a FAP alpha monomer and a DPPIV/CD26 monomer.

94. The composition of claim 77 or 78, wherein the FAP alpha dimer enzyme comprises an amino acid substitution relative to wild type FAP alpha dimer.

95. The composition of claim 77 or 78, wherein the amino acid substitution is present in the β-propeller domain.

96. The composition of claim 95, wherein the amino acid substitution is at Y124, A207, A347, G349, F351, V352.

97. The composition of claim 95, wherein the amino acid substitution is selected from the group consisting of Y124H, A207S, A347V, G349R, F351R, V352P.

98. The composition of claim 77 or 78, wherein the amino acid substitution is present in the catalytic domain.

99. The composition of claim 94, wherein the amino acid substitution is selected from the group consisting of Y124H, A207S, A347V, G349R, F351R, V352P and A657D.

100. The composition of claim 96, wherein the amino acid substitution is at A657.

101. The composition of claim 100, wherein the amino acid substitution is A657D.

102. The composition of claim 94, wherein the amino acid substitution is Y124H or A207S.

103. The composition of claim 94, wherein the amino acid substitution is A347V, G349R, F351R or V352P.

104. The composition of claim 94, wherein the amino acid substitution is present in the entrance to the catalytic domain.

105. The composition of claim 104, wherein the entrance to the catalytic domain is an apical entrance.

106. The composition of claim 105, wherein the amino acid substitution is selected from the group consisting of G64D, Q65H, V299A, D301Q, T354E, V356H, S358T, Y359L, F401E, R402A, V403L, Q405S, T452S, A453V, D457K and Y458E.

107. The composition of claim 104, wherein the entrance is a side entrance.

108. The composition of claim 104, wherein the amino acid substitution is selected from the group consisting of N49K, G50N, F52Y, S53R, Y54L, T56L, F57Y, F58S, P59L, S71Q, D73E, S91E, R93S, M95F, K96D, S97E, V98F, N99G, A100H, S116Y, D117N, S119V, L121Q and Y124H.

109. The composition of claim 94, wherein the amino acid substitution is present at an N-linked glycosylation site.

110. The composition of claim 94, wherein the N-linked glycosylation site is selected from the group consisting of N49, N92, N99, N227, N314 and N679.

111. The composition of claim 110, wherein the amino acid substitution is T229M.

112. The composition of claim 109, 110 or 111, wherein the FAP alpha dimer enzyme is soluble.

113. The composition of claim 94, wherein the amino acid substitution alters disulfide bond formation.

114. The composition of claim 113, wherein the amino acid substitution introduces a disulfide bond.

115. The composition of claim 114, wherein the amino acid substitution is selected from the group consisting of H378C and A386C.

116. The composition of claim 114, wherein the amino acid substitution is selected from the group consisting of L48C, N742C, M683C and I713C.

117. The composition of claim 113, wherein the amino acid substitution removes a disulfide bond.

118. The composition of claim 77 or 78, wherein the FAP alpha dimer enzyme is PEGylated.

119. The composition of claim 77 or 78, wherein the FAP alpha dimer enzyme is a dimerization domain mutant.

120. The composition of claim 119, wherein the dimerization domain mutant lacks residues P232-I250 of wild type FAP alpha dimer enzyme and comprises residues P234-V254 of wild type DPPIV.

121. The composition of claim 119, wherein the dimerization domain mutant lacks residues F706-D731 of wild type FAP alpha dimer enzyme and comprises residues F713-D738 of wild type DPPIV.

122. The composition of claim 119, wherein the dimerization domain mutant comprises an amino acid substitution of T248C.

123. The composition of claim 77, 78 or 84, wherein the FAP alpha dimer enzyme lacks residues N679-N733 from wild type FAP alpha dimer enzyme and comprises residues N685-D739 of wild type DPPIV.

124. The composition of claim 94, wherein the amino acid substitution is present in the cytoplasmic domain.

125. The composition of claim 94, wherein the amino acid substitution is present in the transmembrane domain.

126. The composition of claim 77, 78 or 83, wherein the FAP alpha dimer enzyme lacks a cytoplasmic domain.

127. The composition of claim 77, 78 or 83, wherein the FAP alpha dimer enzyme lacks a transmembrane domain.

128. The composition of claim 77, 78 or 83, wherein the FAP alpha dimer enzyme lacks a cytoplasmic and transmembrane domain.

129. The composition of claim 77 or 78, wherein the FAP alpha dimer enzyme lacks residues corresponding to 1-37 from wild type FAP alpha dimer enzyme.

130. The composition of claim 77 or 78, wherein the FAP alpha dimer enzyme is soluble.

131. The composition of claim 130, wherein the FAP alpha dimer enzyme comprises an amino acid substitution of T229M.

132. The composition of claim 77 or 78, wherein the FAP alpha dimer enzyme comprises an amino acid sequence of SEQ ID NO: 4.

133. The composition of claim 77 or 78, wherein FAP alpha dimer enzyme is present in an amount effective to down-regulate an immune response.

134. The composition comprising

a FAP alpha dimer enzyme comprising an amino acid sequence of SEQ ID NO: 61 or SEQ ID NO: 70, and optionally

(1) one or more amino acid substitutions selected from the group consisting of Y124H, A207S, A347V, G349R, F351R, V352P, A657D, Q732E, N733D, G64D, Q65H, V299A, D301Q, T354E, V356H, S358T, Y359L, F401E, R402A, V403L, Q405S, T452S, A453V, D457K, Y458E, N49K, G50N, F52Y, S53R, Y54L, T56L, F57Y, F58S, P59L, S71Q, D73E, S91E, R93S, M95F, K96D, S97E, V98F, N99G, A100H, S116Y, D117N, S119V, L121Q, Y124H, H378C, A386C, L48C, N742C, M683C, I713C and T248C,

(2) lacking residues P232-I250 and comprising residues P234-V254 of wild type DPPIV,

(3) lacking residues F706-D731 and comprising residues F713-D738 of wild type DPPIV,

(4) lacking residues N679-N733 and comprising residues N685-D739 of wild type DPPIV, or

(5) an amino acid substitution of T229M.

135. The composition of claim 134, wherein the first 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 N-terminal amino acids in SEQ ID NO: 61 are deleted.

136. The composition of claim 135, wherein the FAP alpha dimer enzyme is a fusion or chimeric protein.

137. The composition of claim 136, wherein the fusion or chimeric protein comprises a sequence selected from the group consisting of a secretion sequence, a purification sequence, an epitope, a linker, a protein degradation sequence, a protease cleavage site, a self-cleaving affinity tag, a tissue localization sequence and a peptide or protein ligand.

138. The composition of claim 137, wherein the secretion sequence is a G-CSF leader sequence or an Ig-kappa leader sequence.

139. The composition of claim 137, wherein the purification sequence is selected from the group consisting of a GST sequence tag, a hexahistidine or polyhistidine tag, a Protein A tag, a biotin tag, a chitin tag, and a maltose binding domain.

140. The composition of claim 137, wherein the epitope is selected from the group consisting of a hemaglutinnin tag, a FLAG tag, a V5 tag, a myc tag and a T7 tag.

141. The composition of claim 137, wherein the protein degradation sequence is a PEST sequence.

142. The composition of claim 137, wherein the protease cleavage site is selected from the group consisting of enterokinase, factor Xa protease, thrombin, TEV protease, PreScission protease, Furin, and Genenase.

143. The composition of claim 134 or 135, wherein the FAP alpha dimer enzyme is a heterodimer.

144. The composition of claim 143, wherein the heterodimer is a heterodimer of a FAP alpha monomer and a DPPIV/CD26 monomer.

145. The composition of claim 134 or 135, wherein the amino acid substitution is A657D.

146. The composition of claim 134 or 135, wherein the amino acid substitution is Y124H or A207S.

147. The composition of claim 134 or 135, wherein the amino acid substitution is A347V, G349R, F351R or V352P.

148. The composition of claim 134 or 135, wherein the amino acid substitution is selected from the group consisting of G64D, Q65H, V299A, D301Q, T354E, V356H, S358T, Y359L, F401E, R402A, V403L, Q405S, T452S, A453V, D457K and Y458E.

149. The composition of claim 134 or 135, wherein the amino acid substitution is selected from the group consisting of N49K, G50N, F52Y, S53R, Y54L, T56L, F57Y, F58S, P59L, S71Q, D73E, S91E, R93S, M95F, K96D, S97E, V98F, N99G, A100H, S116Y, D117N, S119V, L121Q and Y124H.

150. The composition of claim 134 or 135, wherein the FAP alpha dimer enzyme is soluble.

151. The composition of claim 134 or 135, wherein the FAP alpha dimer enzyme lacks residues P232-I250 and comprises residues P234-V254 of wild type DPPIV.

152. The composition of claim 134 or 135, wherein the dimerization domain mutant lacks residues F706-D731 and comprises residues F713-D738 of wild type DPPIV.

153. The composition of claim 134 or 135, wherein the FAP alpha dimer enzyme lacks residues N679-N733 and comprises residues N685-D739 of wild type DPPIV.

154. A composition comprising

a FAP alpha dimer enzyme comprising an amino acid substitution of A657D.

155. The composition of claim 154, wherein the FAP alpha dimer enzyme is soluble.

156. The composition of claim 154, wherein the FAP alpha dimer enzyme further comprises an amino acid substitution of T229M.

157. The composition of claim 154, wherein the FAP alpha dimer enzyme further comprises an amino acid substitution of Y124H or A207S.

158. The composition of claim 154, wherein the FAP alpha dimer enzyme further comprises an amino acid substitution of A347V, G349R, F351R or V352P.

159. The composition of claim 154, wherein the FAP alpha dimer enzyme is a fusion or chimeric protein.

160. The composition of claim 159, wherein the fusion or chimeric protein comprises a sequence selected from the group consisting of a secretion sequence, a purification sequence, an epitope, a linker, a protein degradation sequence, a protease cleavage site, a self-cleaving affinity tag, a tissue localization sequence and a peptide or protein ligand.

161. The composition of claim 160, wherein the secretion sequence is a G-CSF leader sequence or an Ig-kappa leader sequence.

162. The composition of claim 160, wherein the purification sequence is selected from the group consisting of a GST sequence tag, a hexahistidine or polyhistidine tag, a Protein A tag, a biotin tag, a chitin tag, and a maltose binding domain.

163. The composition of claim 160, wherein the epitope is selected from the group consisting of a hemaglutinnin tag, a FLAG tag, a V5 tag, a myc tag and a T7 tag.

164. The composition of claim 160, wherein the protein degradation sequence is a PEST sequence.

165. The composition of claim 160, wherein the protease cleavage site is selected from the group consisting of enterokinase, factor Xa protease, thrombin, TEV protease, PreScission protease, Furin, and Genenase.

166. The composition of claim 160, wherein the FAP alpha dimer enzyme is a heterodimer.

167. The composition of claim 166, wherein the heterodimer is a heterodimer of a FAP alpha monomer and a DPPIV/CD26 monomer.

168. The composition of claim 160, wherein the amino acid substitution is A347V, G349R, F351R or V352P.

169. The composition of claim 160, wherein the FAP alpha dimer enzyme further comprises an amino acid substitution of G64D, Q65H, V299A, D301Q, T354E, V356H, S358T, Y359L, F401E, R402A, V403L, Q405S, T452S, A453V, D457K or Y458E.

170. The composition of claim 160, wherein the FAP alpha dimer enzyme further comprises an amino acid substitution of N49K, G50N, F52Y, S53R, Y54L, T56L, F57Y, F58S, P59L, S71Q, D73E, S91E, R93S, M95F, K96D, S97E, V98F, N99G, A100H, S116Y, D117N, S119V, L121Q or Y124H.

171. The composition of claim 160, wherein the FAP alpha dimer enzyme lacks residues P232-I250 and comprises residues P234-V254 of wild type DPPIV.

172. The composition of claim 160, wherein the dimerization domain mutant lacks residues F706-D731 and comprises residues F713-D738 of wild type DPPIV.

173. The composition of claim 160, wherein the FAP alpha dimer enzyme lacks residues N679-N733 and comprises residues N685-D739 of wild type DPPIV.

174. A composition comprising

a FAP alpha dimer enzyme lacking amino acids 269-448 and comprising amino acids 269-448 from mouse FAP.

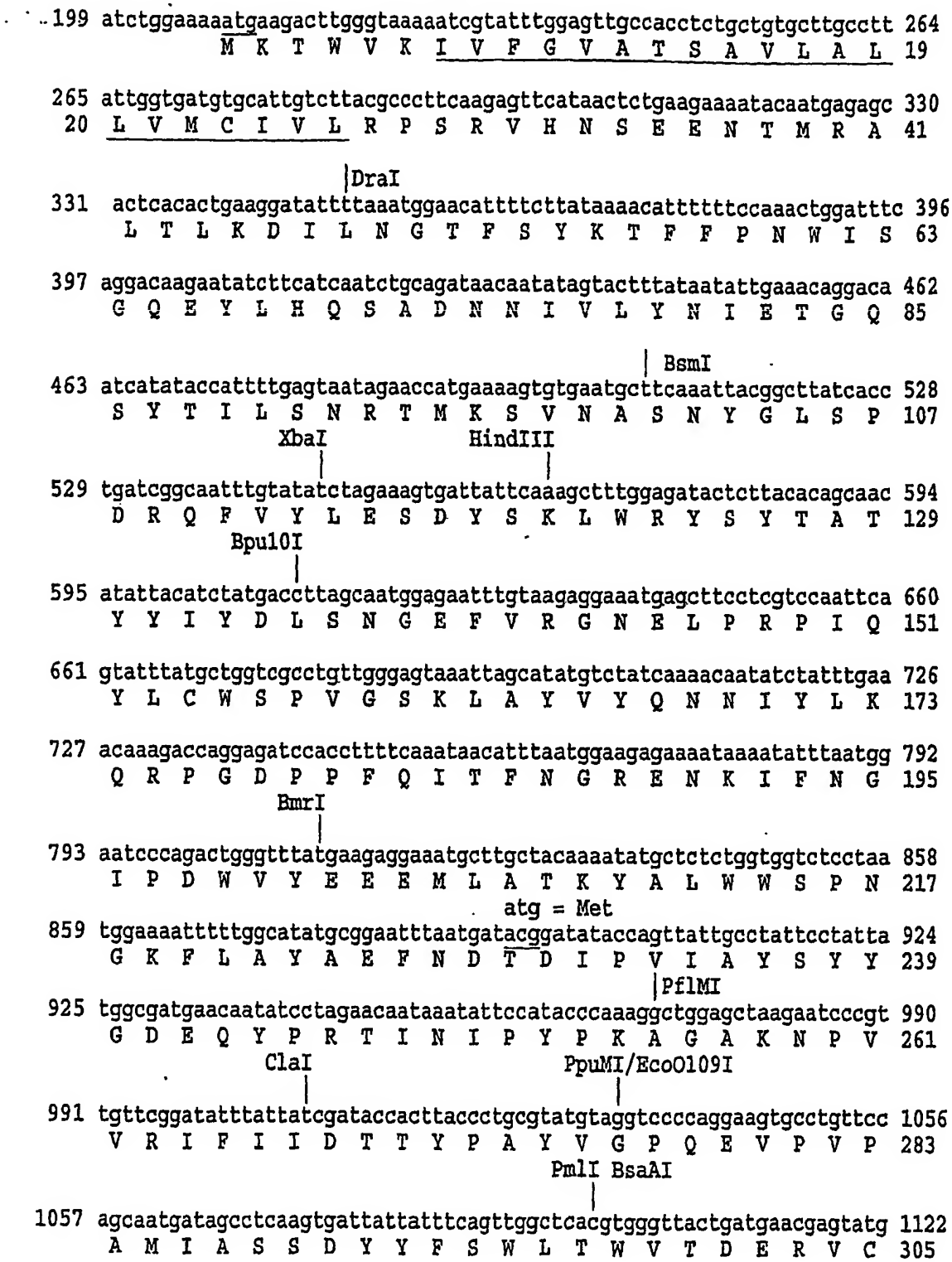

199 atctggaaaaatgaagacttgggtaaaaatcgtatttggagttgccacctctgctgtgcttgcctt 264
M K T W V K I V F G V A T S A V L A L 19
265 attggtgatgtgcattgtcttacgcccttcaagagttcataactctgaagaaaatacaatgagagc 330
20 L V M C I V L R P S R V H N S E E N T M R A 41
DraI
331 actcacactgaaggatatttaaatggaacattttcttataaaacatttttttccaaactggatttc 396
L T L K D I L N G T F S Y K T F F P N W I S 63
397 aggacaagaatatcttcatcaatctgcagataacaatatagtactttataatattgaaacaggaca 462
G Q E Y L H Q S A D N N I V L Y N I E T G Q 85
BsmI
463 atcatataccattttgagtaatagaaccatgaaaagtgtgaatgcttcaaattacggcttatcacc 528
S Y T I L S N R T M K S V N A S N Y G L S P 107
XbaI
HindIII
529 tgatcggcaatttgtatatctagaaagtgattattcaaagctttggagatactcttacacagcaac 594
D R Q F V Y L E S D Y S K L W R Y S Y T A T 129
Bpu10I
595 atattacatctatgaccttagcaatggagaatttgtaagaggaaatgagcttcctcgtccaattca 660
Y Y I Y D L S N G E F V R G N E L P R P I Q 151
661 gtatttatgctggtcgcctgttgggagtaaattagcatatgtctatcaaaacaatatctatttgaa 726
Y L C W S P V G S K L A Y V Y Q N N I Y L K 173
727 acaaagaccaggagatccacctttttcaaataacatttaatggaagagaaaataaaatatttaatgg 792
Q R P G D P P F Q I T F N G R E N K I F N G 195
BmrI
793 aatcccagactgggtttatgaagaggaaatgcttgctacaaaatatgctctctggtggtctcctaa 858
I P D W V Y E E E M L A T K Y A L W W S P N 217
atg = Met
859 tggaaaatttttggcatatgcggaatttaatgatacggatataccagttattgcctattcctatta 924
G K F L A Y A E F N D T D I P V I A Y S Y Y 239
PflMI
925 tggcgatgaacaatatcctagaacaataaatattccatacccaaaggctggagctaagaatcccgt 990
G D E Q Y P R T I N I P Y P K A G A K N P V 261
ClaI
PpuMI/Eco0109I
991 tgttcggatatttattatcgataccacttaccctgcgtatgtaggtccccaggaagtgcctgttcc 1056
V R I F I I D T T Y P A Y V G P Q E V P V P 283
PmlI BsaAI
1057 agcaatgatagcctcaagtgattattatttcagttggctcacgtgggttactgatgaacgagtatg 1122
A M I A S S D Y Y F S W L T W V T D E R V C 305

Fig. 1-1

```
1123 tttgcagtggctaaaaagagtccagaatgtttcggtcctgtctatatgtgacttcagggaagactg 1188
     L  Q  W  L  K  R  V  Q  N  V  S  V  L  S  I  C  D  F  R  E  D  W  327

1189 gcagacatgggattgtccaaagacccaggagcatatagaagaaagcagaactggatgggctggtgg 1254
     Q  T  W  D  C  P  K  T  Q  E  H  I  E  E  S  R  T  G  W  A  G  G  349

1255 attctttgtttcaacaccagttttcagctatgatgccatttcgtactacaaaatatttagtgacaa 1320
     F  F  V  S  T  P  V  F  S  Y  D  A  I  S  Y  Y  K  I  F  S  D  K  371

1321 ggatggctacaaacatattcactatatcaaagacactgtggaaaatgctattcaaattacaagtgg 1386
     D  G  Y  K  H  I  H  Y  I  K  D  T  V  E  N  A  I  Q  I  T  S  G  393

1387 caagtgggaggccataaatatattcagagtaacacaggattcactgttttattctagcaatgaatt 1452
     K  W  E  A  I  N  I  F  R  V  T  Q  D  S  L  F  Y  S  S  N  E  F  415

1453 tgaagaataccctggaagaagaaacatctacagaattagcattggaagctatcctccaagcaagaa 1518
     E  E  Y  P  G  R  R  N  I  Y  R  I  S  I  G  S  Y  P  P  S  K  K  437
                              BanI
                               |
1519 gtgtgttacttgccatctaaggaaagaaaggtgccaatattacacagcaagtttcagcgactacgc 1584
     C  V  T  C  H  L  R  K  E  R  C  Q  Y  Y  T  A  S  F  S  D  Y  A  459
                                                    BspHI
                                                     |
1585 caagtactatgcacttgtctgctacggcccaggcatccccatttccacccttcatgatggacgcac 1650
     K  Y  Y  A  L  V  C  Y  G  P  G  I  P  I  S  T  L  H  D  G  R  T  481
BclI
 |
1651 tgatcaagaaattaaaatcctggaagaaaacaaggaattggaaaatgctttgaaaaatatccagct 1716
     D  Q  E  I  K  I  L  E  E  N  K  E  L  E  N  A  L  K  N  I  Q  L  503

1717 gcctaaagaggaaattaagaaacttgaagtagatgaaattactttatggtacaagatgattcttcc 1782
     P  K  E  E  I  K  K  L  E  V  D  E  I  T  L  W  Y  K  M  I  L  P  525

1783 tcctcaatttgacagatcaaagaagtatcccttgctaattcaagtgtatggtggtccctgcagtca 1848
     P  Q  F  D  R  S  K  K  Y  P  L  L  I  Q  V  Y  G  G  P  C  S  Q  547
                              EcoRV
                                |
1849 gagtgtaaggtctgtatttgctgttaattggatatcttatcttgcaagtaaggaagggatggtcat 1914
     S  V  R  S  V  F  A  V  N  W  I  S  Y  L  A  S  K  E  G  M  V  I  569

1915 tgccttggtggatggtcgaggaacagctttccaaggtgacaaactcctctatgcagtgtatcgaaa 1980
     A  L  V  D  G  R  G  T  A  F  Q  G  D  K  L  L  Y  A  V  Y  R  K  591

1981 gctgggtgtttatgaagttgaagaccagattacagctgtcagaaaattcatagaaatgggtttcat 2046
     L  G  V  Y  E  V  E  D  Q  I  T  A  V  R  K  F  I  E  M  G  F  I  613

2047 tgatgaaaaaagaatagccatatggggctggtcctatggaggatacgtttcatcactggcccttgc 2112
     D  E  K  R  I  A  I  W  G  W  S  Y  G  G  Y  V  S  S  L  A  L  A  635
```

Fig. 1-2

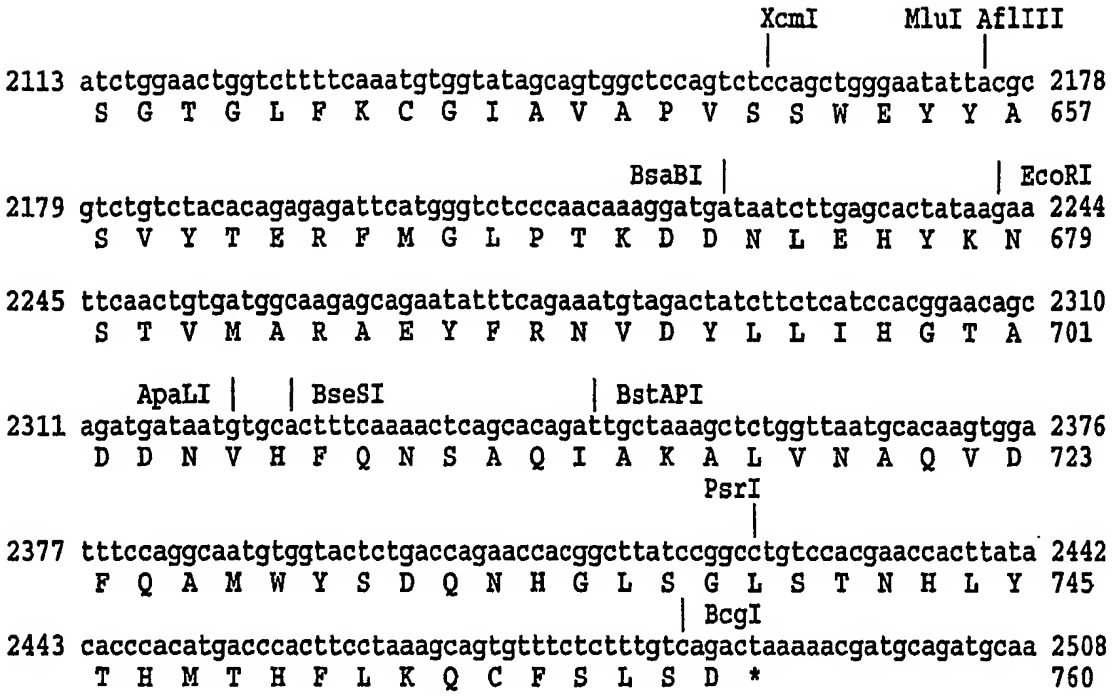

XcmI
MluI AflIII
2113 atctggaactggtcttttcaaatgtggtatagcagtggctccagtctccagctgggaatattacgc 2178
S G T G L F K C G I A V A P V S S W E Y Y A 657
BsaBI
EcoRI
2179 gtctgtctacacagagagattcatgggtctcccaacaaaggatgataatcttgagcactataagaa 2244
S V Y T E R F M G L P T K D D N L E H Y K N 679
2245 ttcaactgtgatggcaagagcagaatatttcagaaatgtagactatcttctcatccacggaacagc 2310
S T V M A R A E Y F R N V D Y L L I H G T A 701
ApaLI
BseSI
BstAPI
2311 agatgataatgtgcactttcaaaactcagcacagattgctaaagctctggttaatgcacaagtgga 2376
D D N V H F Q N S A Q I A K A L V N A Q V D 723
PsrI
2377 tttccaggcaatgtggtactctgaccagaaccacggcttatccggcctgtccacgaaccacttata 2442
F Q A M W Y S D Q N H G L S G L S T N H L Y 745
BcgI
2443 cacccacatgacccacttcctaaagcagtgtttctctttgtcagactaaaaacgatgcagatgcaa 2508
T H M T H F L K Q C F S L S D * 760

Fig. 1-3

```
hFAP:     1  MKTWVKIVFGV-ATSAVLALLVMCIVLRPSRVHNSEENTMRALTLKDILNGTFSYKTFFP 59
             MKT  K++ G+   +A++ ++ + +VL      ++  ++ +  TL D L  T+  K +
hDPPIV:   1  MKTPWKVLLGLLGAAALVTIITVPVVLLNKGTDDATADSRKTYTLTDYLKNTYRLKLYSL 60

hFAP:    60  NWISGQEYLHQSADNNIVLYNIETGQSYTILSNRTMKSVNAS--NYGLSPDRQFVYLESD 117
              WIS  EYL++  +NNI+++N E G S   L N T      S  +Y +SPD QF+ LE +
hDPPIV:  61  RWISDHEYLYKQ-ENNILVFNAEYGNSSVFLENSTFDEFGHSINDYSISPDGQFILLEYN 119

hFAP:   118  YSKLWRYSYTATYYIYDLSNGEFVRGNELPRPIQYLCWSPVGSKLAYVYQNNIYLKQRPG 177
             Y K WR+SYTA+Y IYDL+  + +    +P   Q++ WSPVG KLAYV+ N+IY+K  P
hDPPIV:120  YVKQWRHSYTASYDIYDLNKRQLITEERIPNNTQWVTWSPVGHKLAYVWNNDIYVKIEPN 179

hFAP:   178  DPPFQITFNGRENKIFNGIPDWVYEEEMLATKYALWWSPNGKFLAYAEFNDTDIPVIAYS 237
              P ++IT+ G+E+ I+NGI DWVYEEE+ +   ALWWSPNG FLAYA+FNDT++P+I YS
hDPPIV:180  LPSYRITWTGKEDIIYNGITDWVYEEEVFSAYSALWWSPNGTFLAYAQFNDTEVPLIEYS 239

hFAP:   238  YYGDE--QYPRTINIPYPKAGAKNPVVRIFIIDTTYPAYVGPQ---EVPVPAMIASSDYY 292
             +Y DE  QYP+T+ +PYPKAGA NP V+ F+++T   + V      ++  PA +   D+Y
hDPPIV:240  FYSDESLQYPKTVRVPYPKAGAVNPTVKFFVVNTDSLSSVTNATSIQITAPASMLIGDHY 299

hFAP:   293  FSWLTWVTDERVCLQWLKRVQNVSVLSICDFREDWQTWDCPKTQEHIEESRTGWAGGFFV 352
                +TW T ER+ LQWL+R+QN SV+ ICD+ E    W+C   ++HIE S TGW G F
hDPPIV:300  LCDVTWATQERISLQWLRRIQNYSVMDICDYDESSGRWNCLVARQHIEMSTTGWVGRFRP 359

hFAP:   353  STPVFSYDAISYYKIFSDKDGYKHIHYIKDTVENAIQITSGKWEAINIFRVTQDSLFYSS 412
             S P F+ D S+YKI S+++GY+HI Y +   ++   IT G WE I I  +T D L+Y S
hDPPIV:360  SEPHFTLDGNSFYKIISNEEGYRHICYFQIDKKDCTFITKGTWEVIGIEALTSDYLYYIS 419

hFAP:   413  NEFEEYPGRRNIYRISIGSYPPSKKCVTCHLRKERCQYYTASFSDYAKYYALVCYGPGIP 472
             NE++  PG RN+Y+I +  Y     C++C L  ERCQYY+ SFS  AKYY L C GPG+P
hDPPIV:420  NEYKGMPGGRNLYKIQLSDYT-KVTCLSCELNPERCQYYSVSFSKEAKYYQLRCSGPGLP 478

hFAP:   473  ISTLHDGRTDQEIKILEENKELENALKNIQLPKEEIKKLEVDEITLWYKMILPPQFDRSK 532
             + TLH    D+ +++LE+N  L+  L N+Q+P +++  + ++E   WY+MILPP FD+SK
hDPPIV:479  LYTLHSSVNDKGLRVLEDNSALDKMLQNVQMPSKKLDFIILNETKFWYQMILPPHFDKSK 538

hFAP:   533  KYPLLIQVYGGPCSQSVRSVFAVNWISYLASKEGMVIALVDGRGTAFQGDKLLYAVYRKL 592
             KYPLL+ VY GPCSQ   +VF +NW +YLAS E +++A  DGRG+ +QGDK+++A+ R+L
hDPPIV:539  KYPLLLDVYAGPCSQKADTVFRLNWATYLASTENIIVASFDGRGSGYQGDKIMHAINRRL 598

hFAP:   593  GVYEVEDQITAVRKFIEMGFIDEKRIAIWGWSYGGYVSSLALASGTGLFKCGIAVAPVSS 652
             G +EVEDQI A R+F +MGF+D KRIAIWGWSYGGYV+S+ L SG+G+FKCGIAVAPVS
hDPPIV:599  GTFEVEDQIEAARQFSKMGFVDNKRIAIWGWSYGGYVTSMVLGSGSGVFKCGIAVAPVSR 658

hFAP:   653  WEYYASVYTERFMGLPTKDDNLEHYKNSTVMARAEYFRNVDYLLIHGTADDNVHFQNSAQ 712
             WEYY SVYTER+MGLPT +DNL+HY+NSTVM+RAE F+ V+YLLIHGTADDNVHFQ SAQ
hDPPIV:659  WEYYDSVYTERYMGLPTPEDNLDHYRNSTVMSRAENFKQVEYLLIHGTADDNVHFQQSAQ 718

hFAP:   713  IAKALVNAQVDFQAMWYSDQNHGL-SGLSTNHLYTHMTHFLKQCFSLSD 760
             I+KALV+  VDFQAMWY+D++HG+ S  +  H+YTHM+HF+KQCFSL
hDPPIV:719  ISKALVDVGVDFQAMWYTDEDHGIASSTAHQHIYTHMSHFIKQCFSLP  766
```

Fig. 2

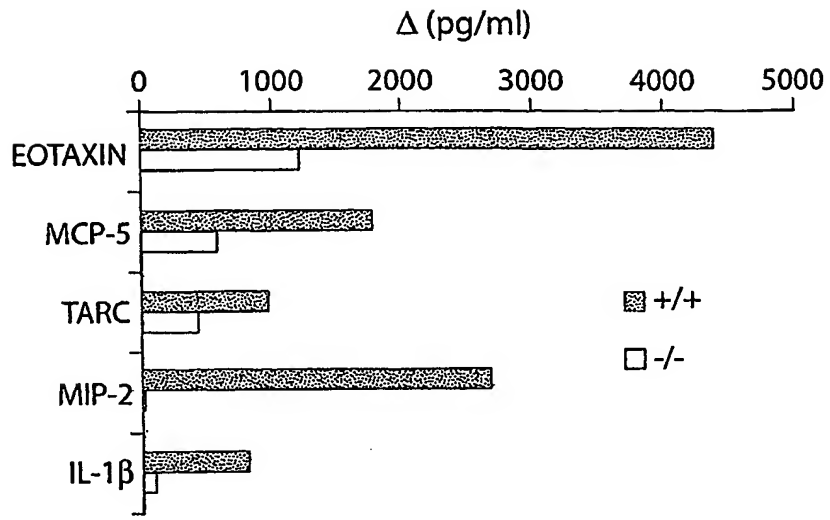

Δ (pg/ml)
0
1000
2000
3000
4000
5000
EOTAXIN
MCP-5
TARC
MIP-2
IL-1β
+/+
-/-

Fig. 3A

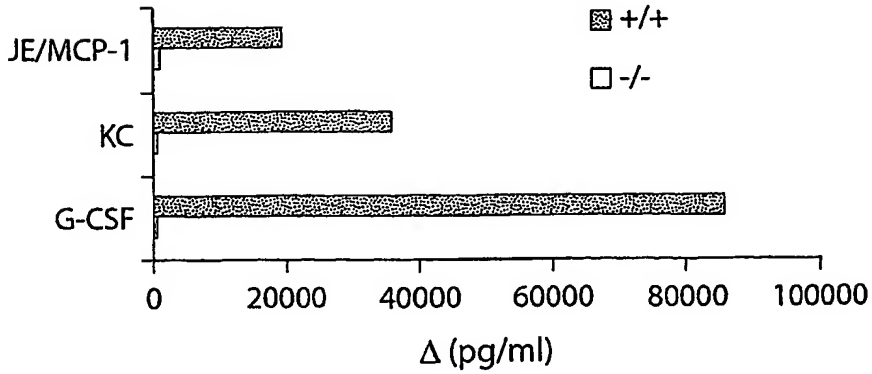

JE/MCP-1
KC
G-CSF
+/+
-/-
0
20000
40000
60000
80000
100000
Δ (pg/ml)

Fig. 3B

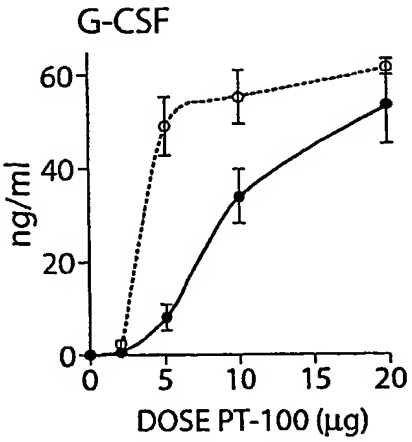


Fig. 4A

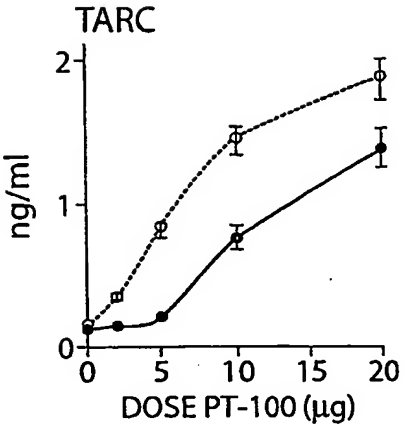


Fig. 4B

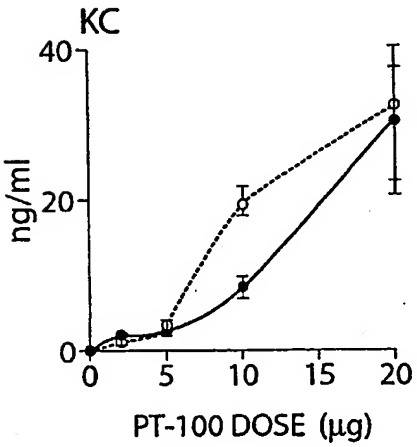


Fig. 4C

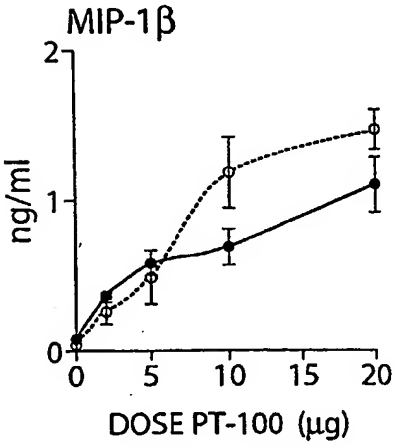


Fig. 4D

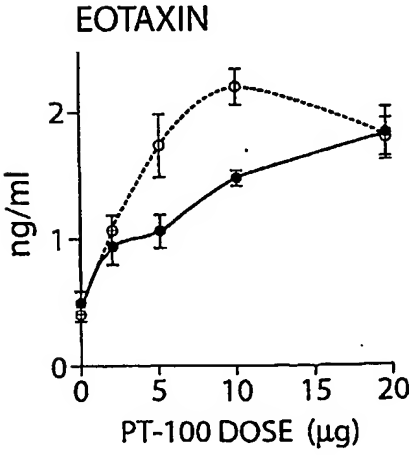


Fig. 4E

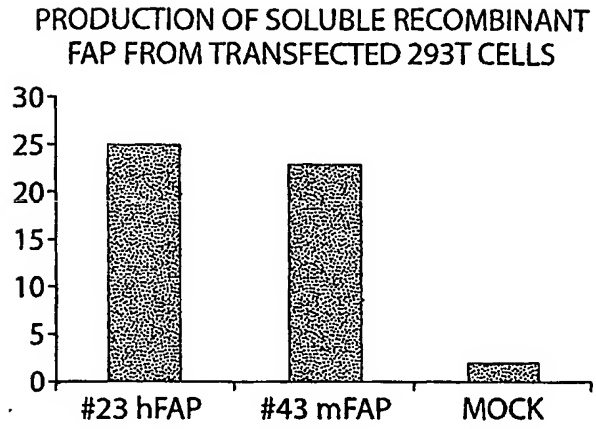

PRODUCTION OF SOLUBLE RECOMBINANT
FAP FROM TRANSFECTED 293T CELLS
30
25
20
15
10
5
0
#23 hFAP
#43 mFAP
MOCK

Fig. 5

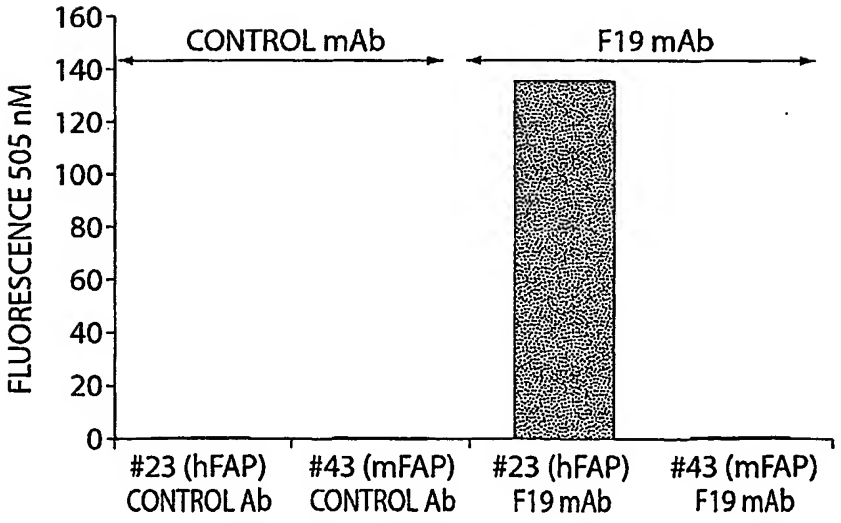

FLUORESCENCE 505 nM
160
140
120
100
80
60
40
20
0
CONTROL mAb
F19 mAb
#23 (hFAP)
CONTROL Ab
#43 (mFAP)
CONTROL Ab
#23 (hFAP)
F19 mAb
#43 (mFAP)
F19 mAb

Fig. 6

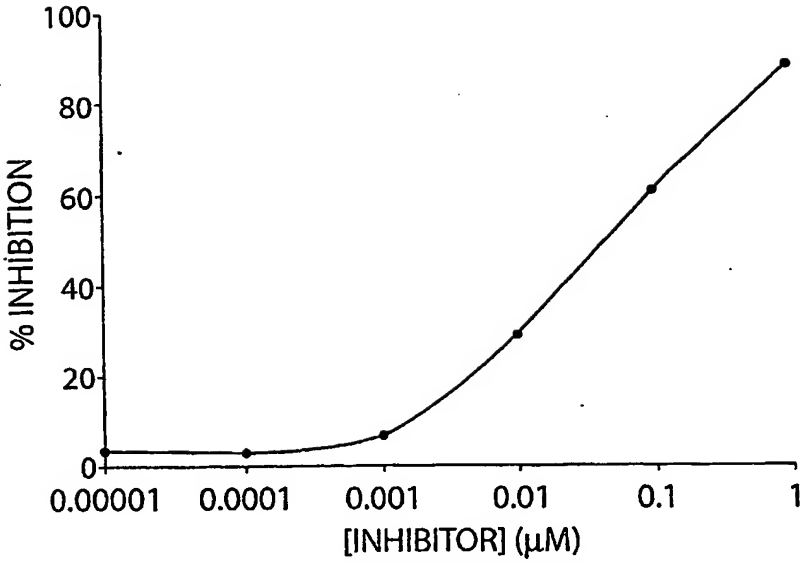

100
80
60
40
20
0
% INHIBITION
0.00001
0.0001
0.001
0.01
0.1
1
[INHIBITOR] (μM)

Fig. 7

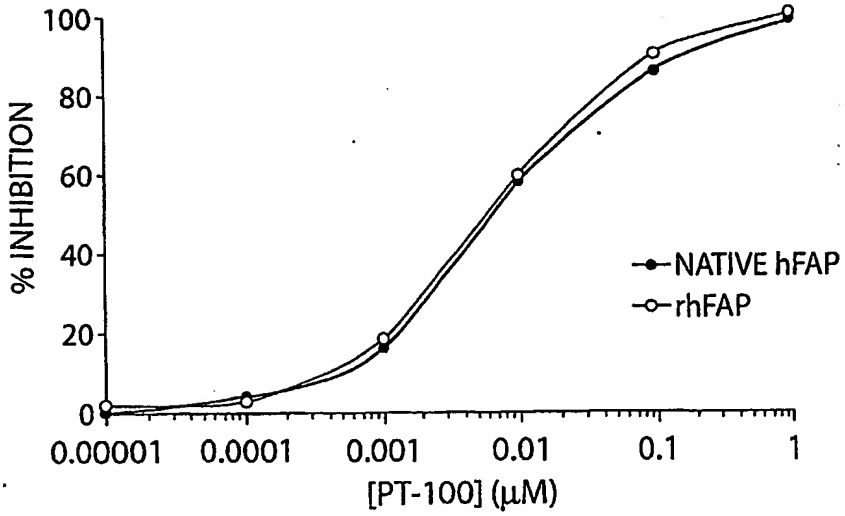

100
80
60
40
20
0
% INHIBITION
0.00001
0.0001
0.001
0.01
0.1
1
[PT-100] (μM)
NATIVE hFAP
rhFAP

Fig. 8

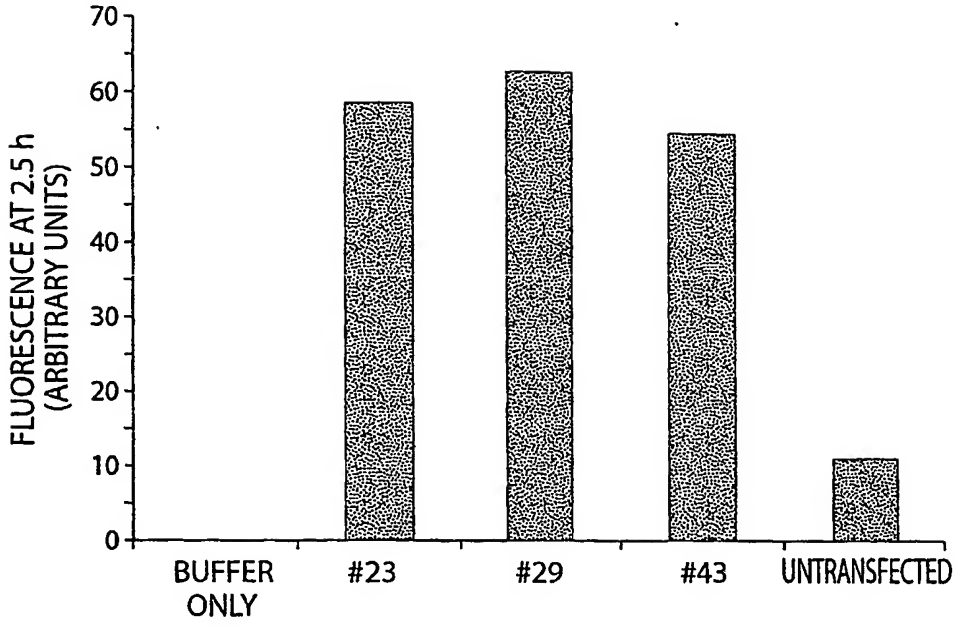

FLUORESCENCE AT 2.5 h
(ARBITRARY UNITS)
70
60
50
40
30
20
10
0
BUFFER ONLY
#23
#29
#43
UNTRANSFECTED

Fig. 9

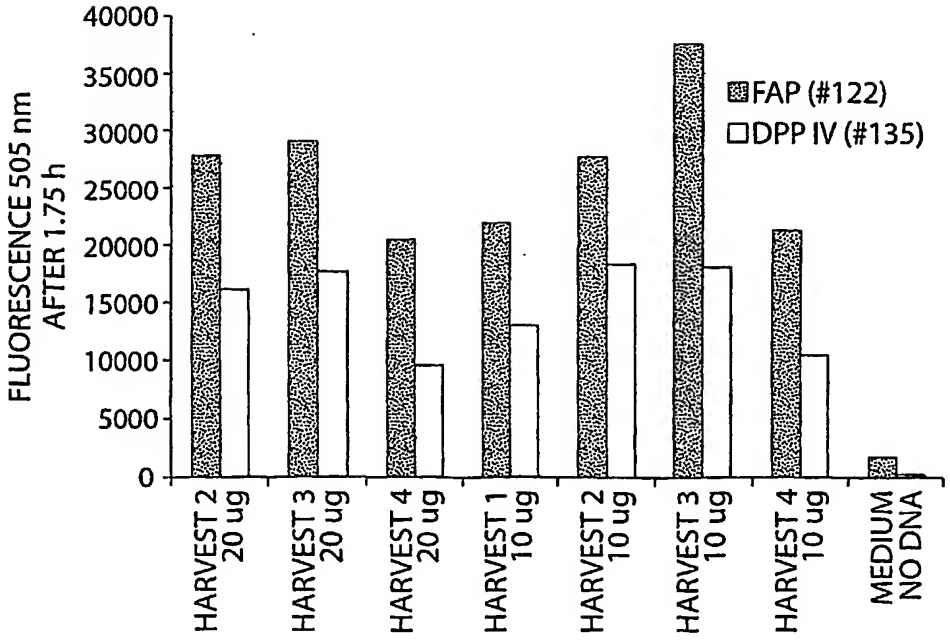

FLUORESCENCE 505 nm
AFTER 1.75 h
40000
35000
30000
25000
20000
15000
10000
5000
0
FAP (#122)
DPP IV (#135)
HARVEST 2 20 ug
HARVEST 3 20 ug
HARVEST 4 20 ug
HARVEST 1 10 ug
HARVEST 2 10 ug
HARVEST 3 10 ug
HARVEST 4 10 ug
MEDIUM NO DNA

Fig. 10

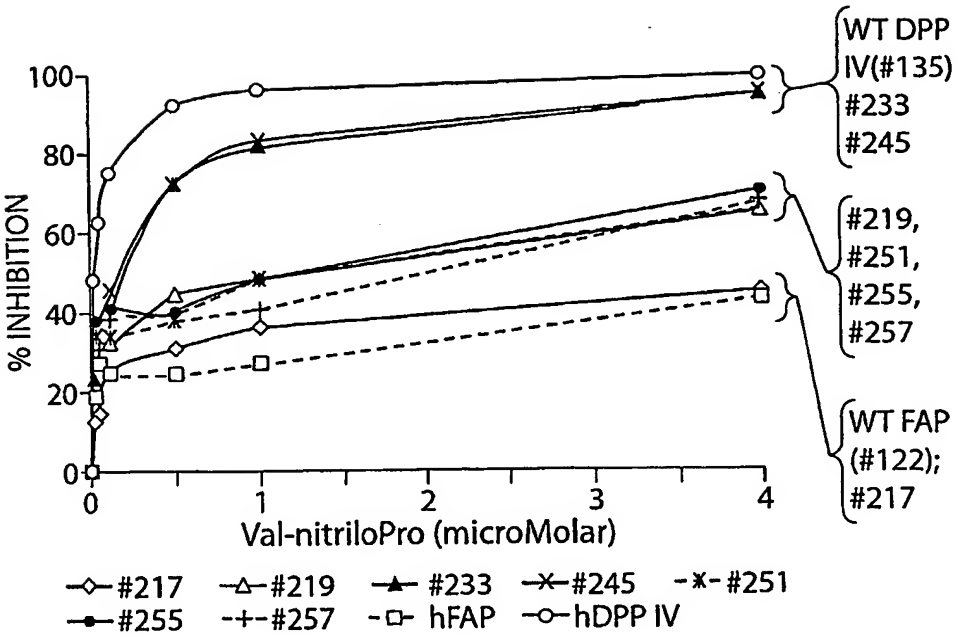

WT DPP IV(#135) #233 #245
#219, #251, #255, #257
WT FAP (#122); #217
% INHIBITION
100
80
60
40
20
0
0
1
2
3
4
Val-nitriloPro (microMolar)
#217 #219 #233 #245 #251
#255 #257 hFAP hDPP IV

Fig. 11

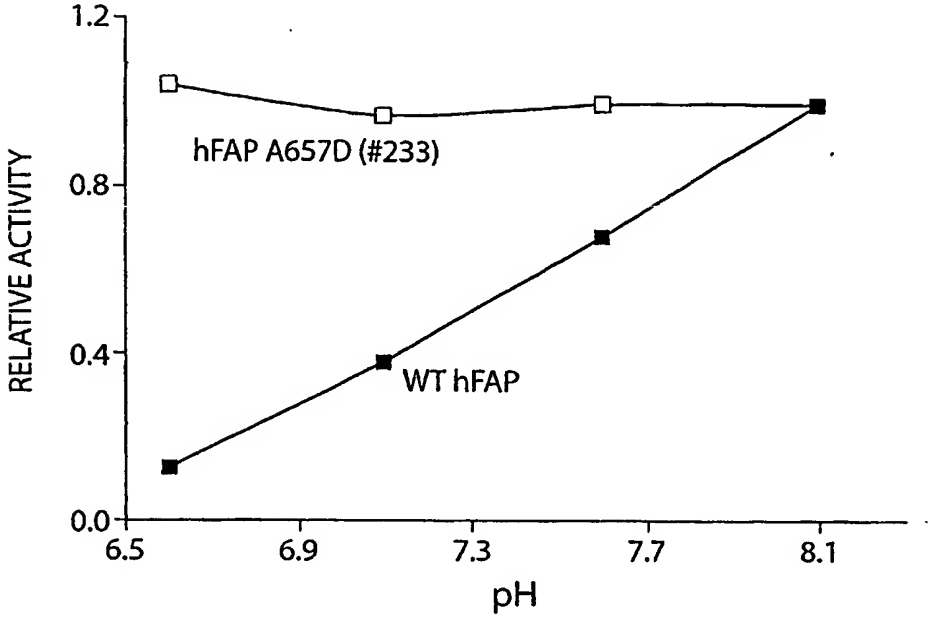


Fig. 12A

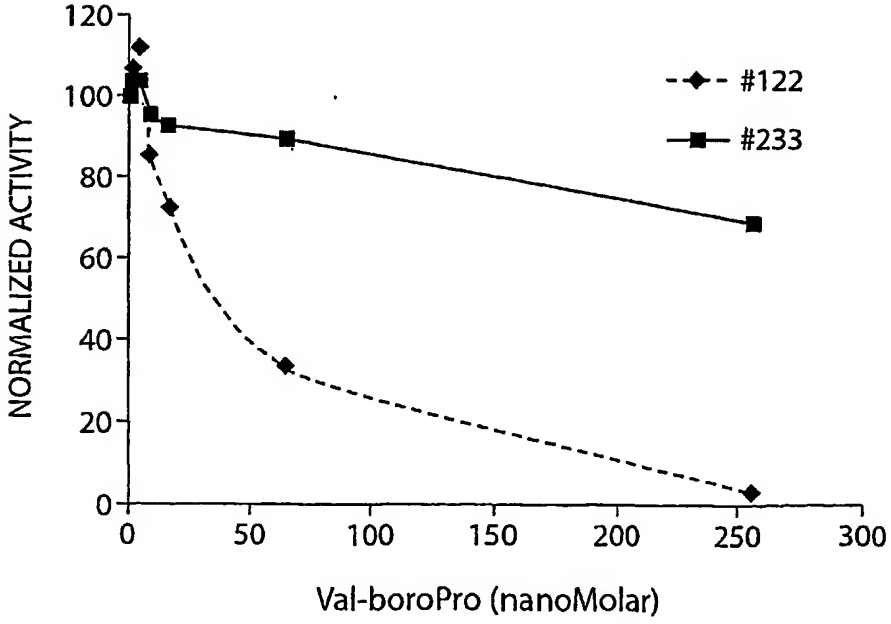


Fig. 12B

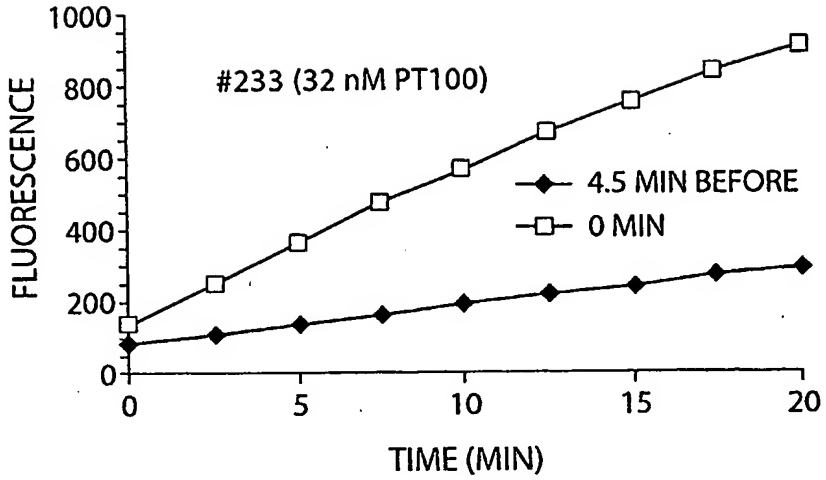


Fig. 12C

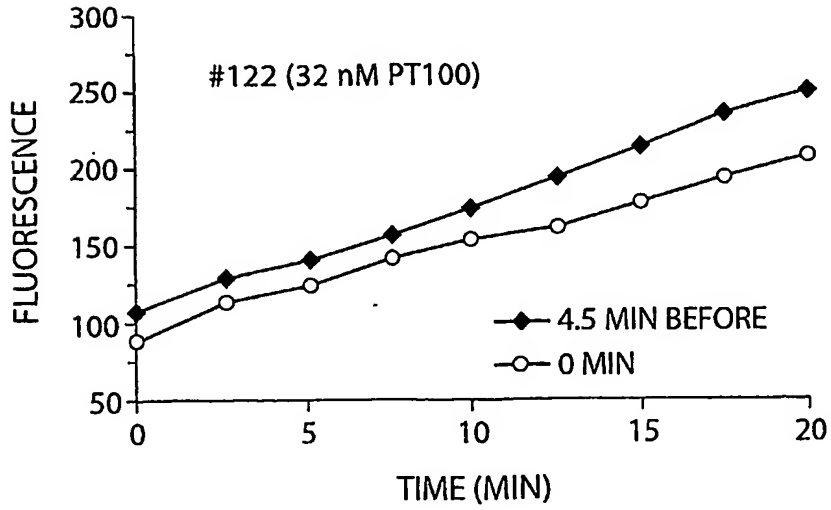


Fig. 12D

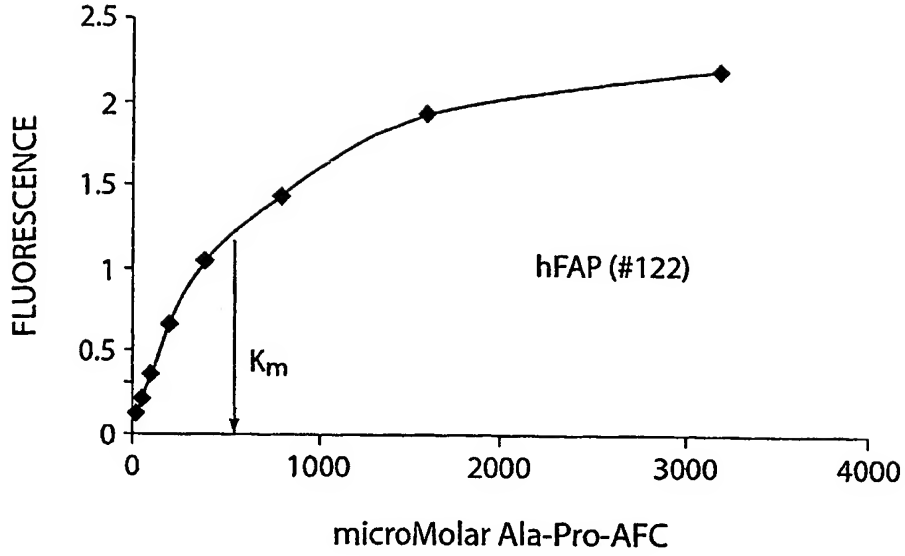


Fig. 12E

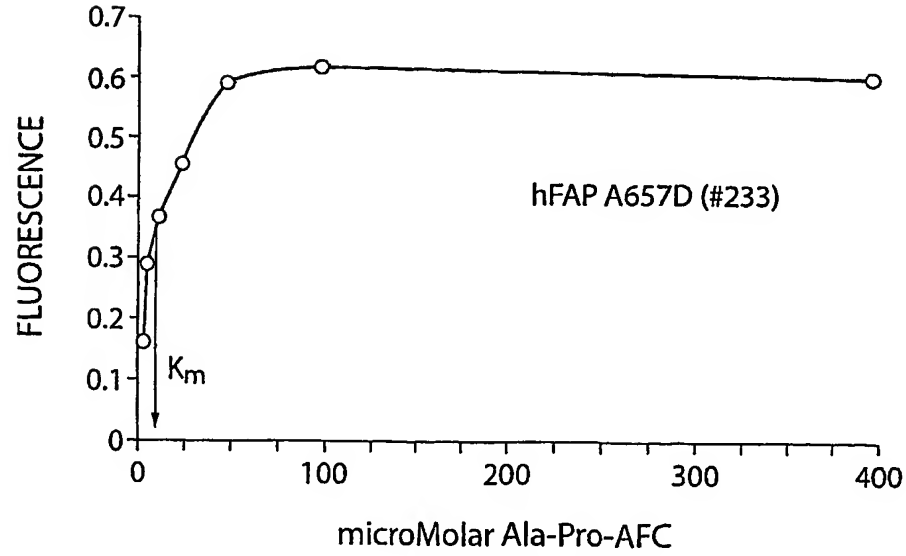


Fig. 12F

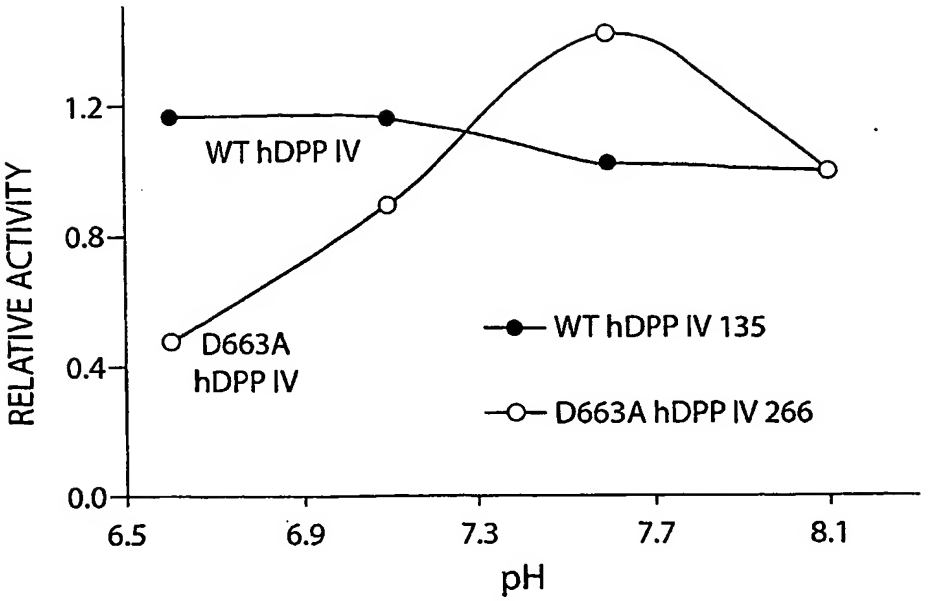


Fig. 13A

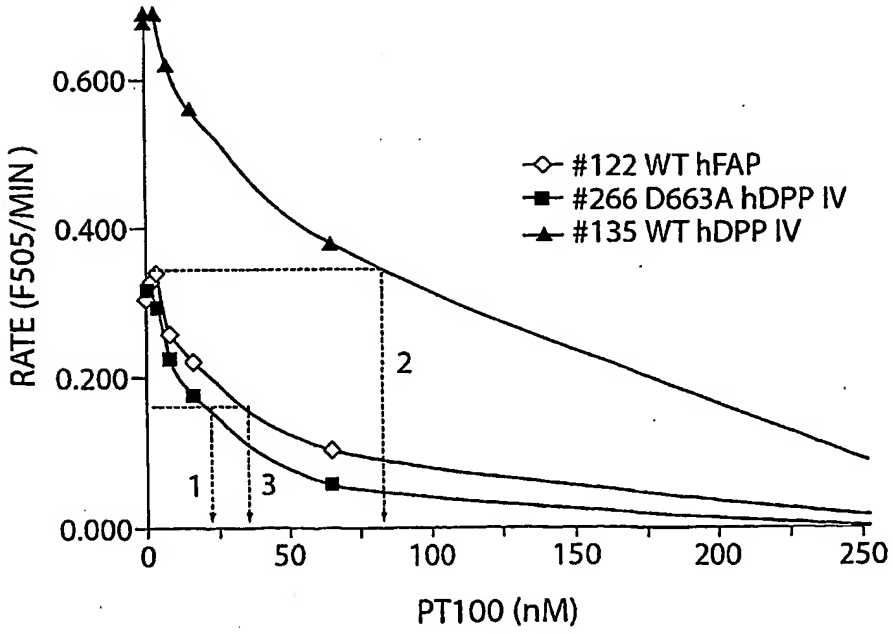


Fig. 13B

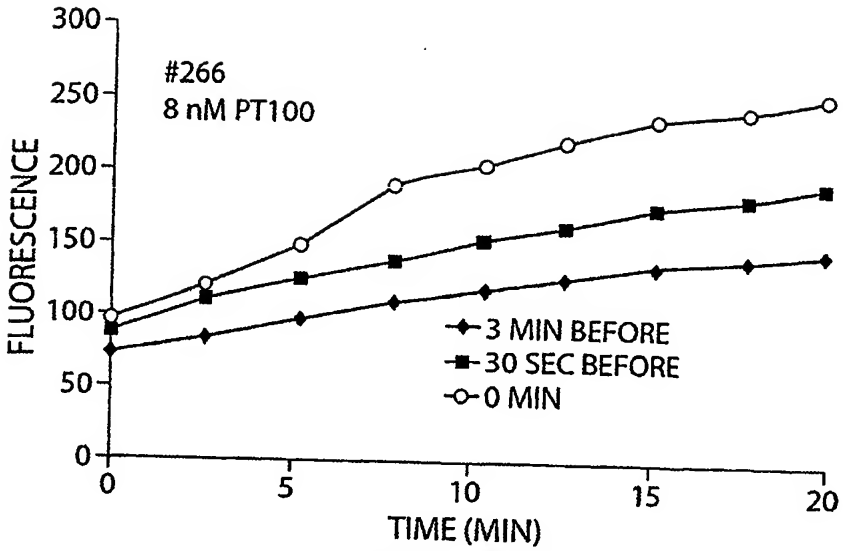


Fig. 13C

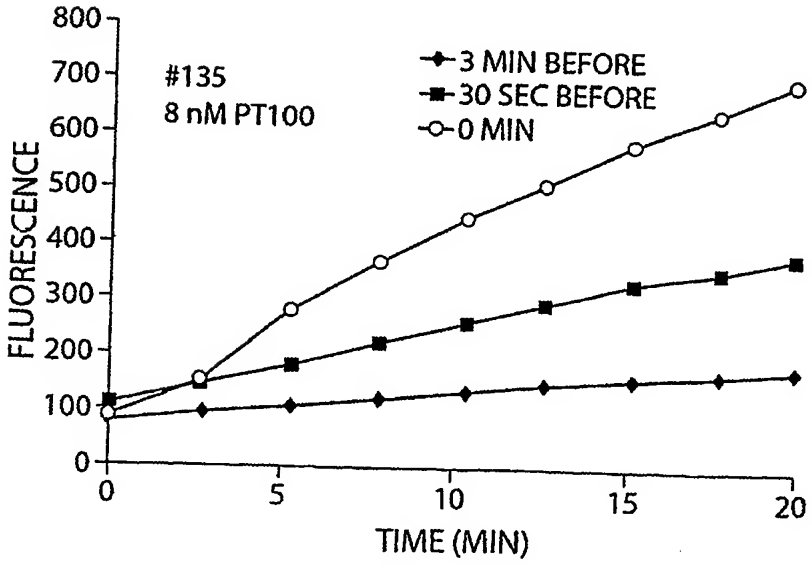


Fig. 13D

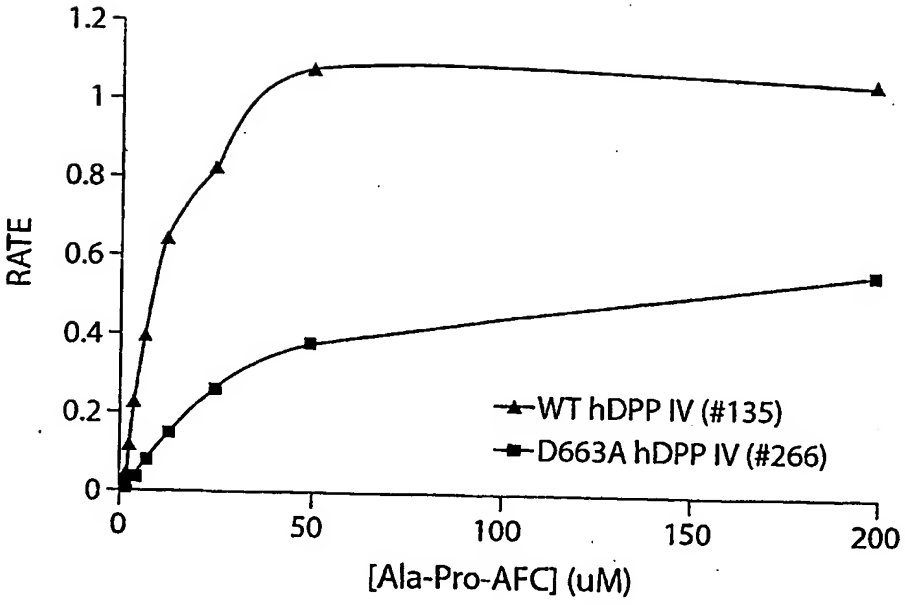

1.2
1
0.8
0.6
0.4
0.2
0
RATE
0
50
100
150
200
[Ala-Pro-AFC] (uM)
WT hDPP IV (#135)
D663A hDPP IV (#266)

Fig. 13E

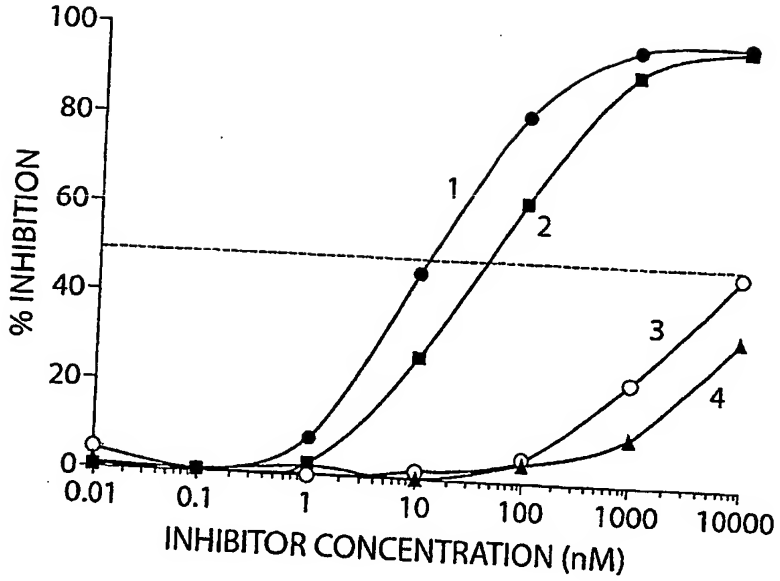

100
80
60
40
20
0
% INHIBITION
1
2
3
4
0.01
0.1
1
10
100
1000
10000
INHIBITOR CONCENTRATION (nM)

Fig. 14

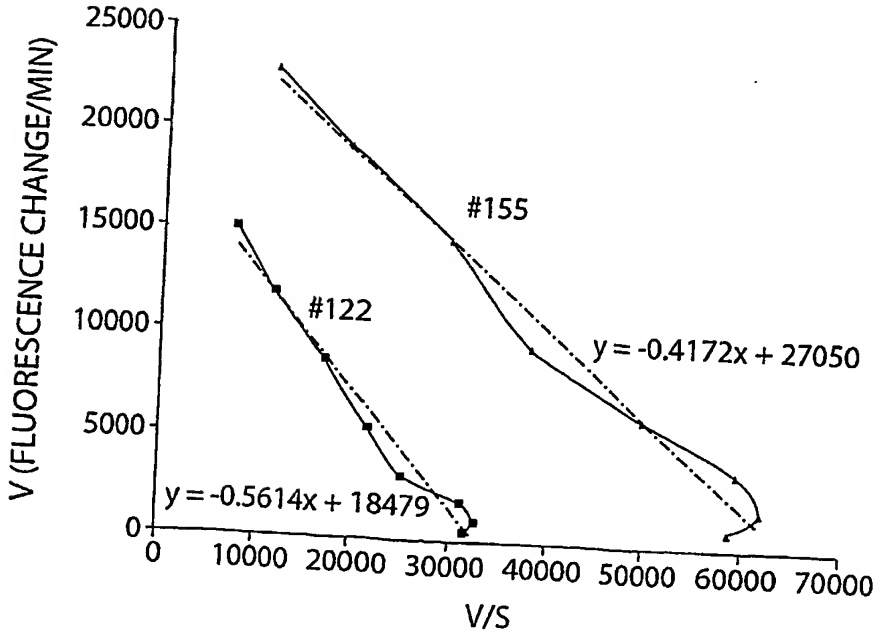

25000
20000
15000
10000
5000
0
V (FLUORESCENCE CHANGE/MIN)
#155
#122
y = -0.4172x + 27050
y = -0.5614x + 18479
0
10000
20000
30000
40000
50000
60000
70000
V/S

Fig. 15

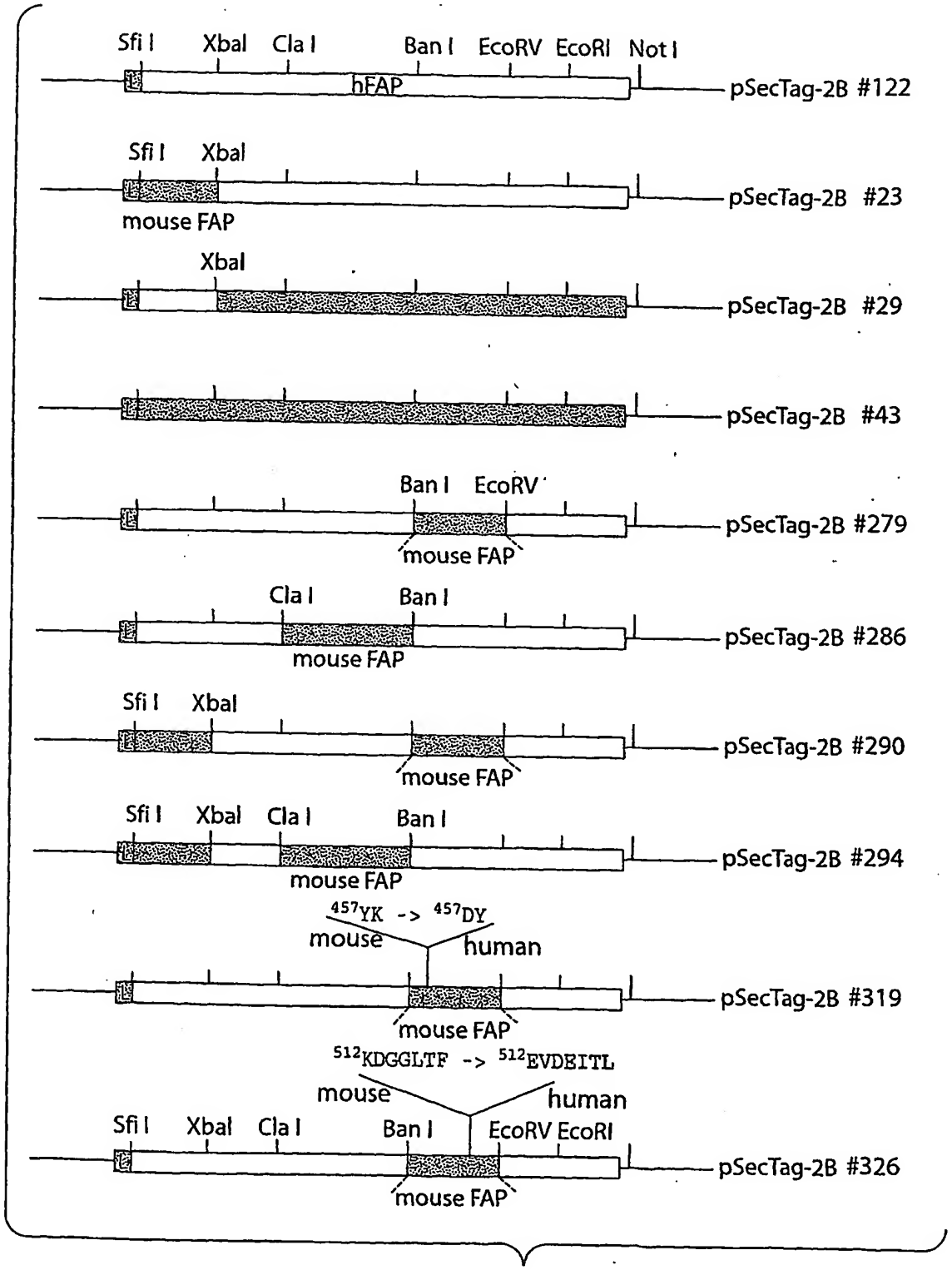

Sfi I
Xbal
Cla I
Ban I
EcoRV
EcoRI
Not I
hFAP
pSecTag-2B #122
Sfi I
Xbal
mouse FAP
pSecTag-2B #23
Xbal
pSecTag-2B #29
pSecTag-2B #43
Ban I
EcoRV
mouse FAP
pSecTag-2B #279
Cla I
Ban I
mouse FAP
pSecTag-2B #286
Sfi I
Xbal
mouse FAP
pSecTag-2B #290
Sfi I
Xbal
Cla I
Ban I
mouse FAP
pSecTag-2B #294
457YK -> 457DY
mouse
human
mouse FAP
pSecTag-2B #319
512KDGGLTF -> 512EVDEITL
mouse
human
Sfi I
Xbal
Cla I
Ban I
EcoRV
EcoRI
mouse FAP
pSecTag-2B #326

Fig. 16A

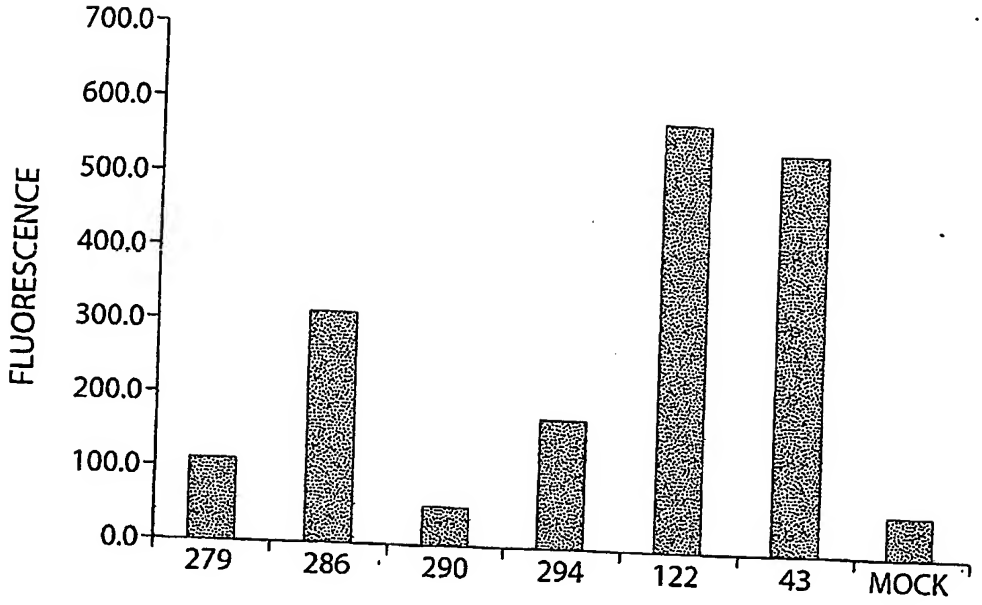


Fig. 16B

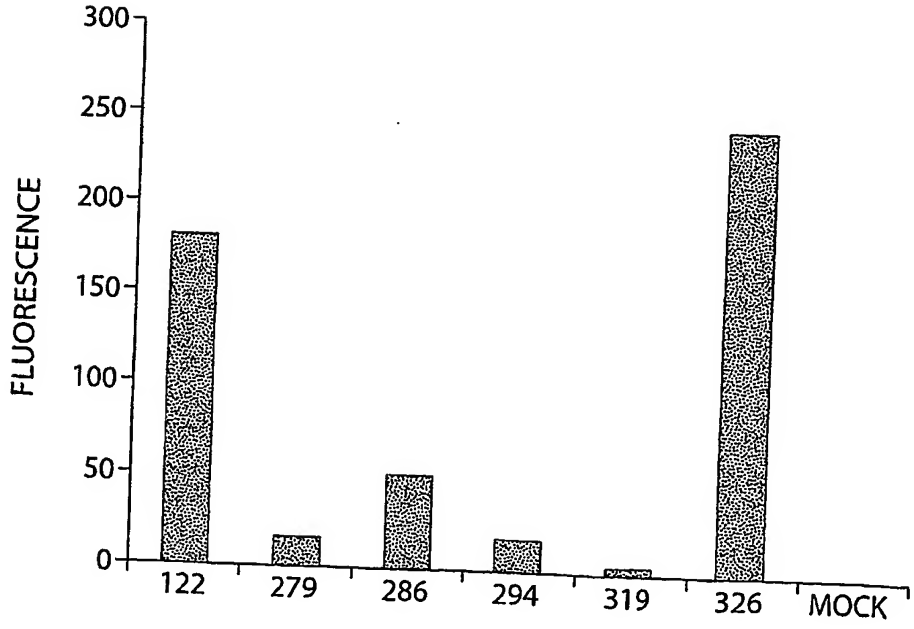


Fig. 16C

SEQUENCE LISTING

<110> Point Therapeutics, Inc.
McLean, Paul A
Jones, Barry
Miller, Glenn T
Jesson, Michael I

<120> FAP COMPOSITIONS AND METHODS OF USE THEREOF FOR IMMUNOMODULATION

<130> I0248.70022WO00

<140> not yet assigned
<141> 2005-01-10

<150> 60/535,577
<151> 2004-01-09

<160> 70

<170> PatentIn version 3.3

<210> 1
<211> 2788
<212> DNA
<213> Homo sapiens

<400> 1
aagaacgccc ccaaaatctg tttctaattt tacagaaatc ttttgaaact tggcacggta 60

ttcaaaagtc cgtggaaaga aaaaaacctt gtcctggctt cagcttccaa ctacaaagac 120

agacttggtc cttttcaacg gttttcacag atccagtgac ccacgctctg aagacagaat 180

tagctaactt tcaaaaacat ctggaaaaat gaagacttgg gtaaaaatcg tatttggagt 240

tgccacctct gctgtgcttg ccttattggt gatgtgcatt gtcttacgcc cttcaagagt 300

tcataactct gaagaaaata caatgagagc actcacactg aaggatattt taaatggaac 360

attttcttat aaaacatttt ttccaaactg gatttcagga caagaatatc ttcatcaatc 420

tgcagataac aatatagtac tttataatat tgaaacagga caatcatata ccattttgag 480

taatagaacc atgaaaagtg tgaatgcttc aaattacggc ttatcacctg atcggcaatt 540

tgtatatcta gaaagtgatt attcaaagct ttggagatac tcttacacag caacatatta 600

catctatgac cttagcaatg gagaatttgt aagaggaaat gagcttcctc gtccaattca 660

gtatttatgc tggtcgcctg ttgggagtaa attagcatat gtctatcaaa acaatatcta 720

tttgaaacaa agaccaggag atccaccttt tcaaataaca tttaatggaa gagaaaataa 780

aatatttaat ggaatcccag actgggttta tgaagaggaa atgcttgcta caaaatatgc 840

tctctggtgg tctcctaatg gaaaattttt ggcatatgcg gaatttaatg atacggatat 900

accagttatt gcctattcct attatggcga tgaacaatat cctagaacaa taaatattcc 960

atacccaaag gctggagcta agaatcccgt tgttcggata tttattatcg ataccactta 1020

ccctgcgtat gtaggtcccc aggaagtgcc tgttccagca atgatagcct caagtgatta 1080

ttatttcagt tggctcacgt gggttactga tgaacgagta tgtttgcagt ggctaaaaag 1140

agtccagaat gtttcggtcc tgtctatatg tgacttcagg gaagactggc agacatggga 1200

ttgtccaaag acccaggagc atatagaaga aagcagaact ggatgggctg gtggattctt 1260

tgtttcaaca ccagttttca gctatgatgc catttcgtac tacaaaatat ttagtgacaa 1320

ggatggctac aaacatattc actatatcaa agacactgtg gaaaatgcta ttcaaattac 1380

aagtggcaag tgggaggcca taaatatatt cagagtaaca caggattcac tgttttattc 1440

tagcaatgaa tttgaagaat accctggaag aagaaacatc tacagaatta gcattggaag 1500

ctatcctcca agcaagaagt gtgttacttg ccatctaagg aaagaaaggt gccaatatta 1560

cacagcaagt ttcagcgact acgccaagta ctatgcactt gtctgctacg gcccaggcat 1620

ccccatttcc acccttcatg atggacgcac tgatcaagaa attaaaatcc tggaagaaaa 1680

caaggaattg gaaaatgctt tgaaaaatat ccagctgcct aaagaggaaa ttaagaaact 1740

tgaagtagat gaaattactt tatggtacaa gatgattctt cctcctcaat ttgacagatc 1800

aaagaagtat cccttgctaa ttcaagtgta tggtggtccc tgcagtcaga gtgtaaggtc 1860

tgtatttgct gttaattgga tatcttatct tgcaagtaag gaagggatgg tcattgcctt 1920

ggtggatggt cgaggaacag ctttccaagg tgacaaactc ctctatgcag tgtatcgaaa 1980

gctgggtgtt tatgaagttg aagaccagat tacagctgtc agaaaattca tagaaatggg 2040

tttcattgat gaaaaaagaa tagccatatg gggctggtcc tatggaggat acgtttcatc 2100

actggccctt gcatctggaa ctggtctttt caaatgtggt atagcagtgg ctccagtctc 2160

cagctgggaa tattacgcgt ctgtctacac agagagattc atgggtctcc caacaaagga 2220

tgataatctt gagcactata agaattcaac tgtgatggca agagcagaat atttcagaaa 2280

tgtagactat cttctcatcc acggaacagc agatgataat gtgcactttc aaaactcagc 2340

acagattgct aaagctctgg ttaatgcaca agtggatttc caggcaatgt ggtactctga 2400

ccagaaccac ggcttatccg gcctgtccac gaaccactta tacacccaca tgacccactt 2460

cctaaagcag tgtttctctt tgtcagacta aaaacgatgc agatgcaagc ctgtatcaga 2520

atctgaaaac cttatataaa cccctcagac agtttgctta ttttattttt tatgttgtaa 2580

aatgctagta taaacaaaca aattaatgtt gttctaaagg ctgttaaaaa aaagatgagg 2640

actcagaagt tcaagctaaa tattgtttac attttctggt actctgtgaa agaagagaaa 2700

agggagtcat gcattttgct ttggacacag tgttttatca cctgttcatt tgaagaaaaa 2760

taataaagtc agaagttcaa aaaaaaaa 2788

<210> 2

<211> 760
<212> PRT
<213> Homo sapiens

<400> 2

Met Lys Thr Trp Val Lys Ile Val Phe Gly Val Ala Thr Ser Ala Val
1 5 10 15

Leu Ala Leu Leu Val Met Cys Ile Val Leu Arg Pro Ser Arg Val His
20 25 30

Asn Ser Glu Glu Asn Thr Met Arg Ala Leu Thr Leu Lys Asp Ile Leu
35 40 45

Asn Gly Thr Phe Ser Tyr Lys Thr Phe Phe Pro Asn Trp Ile Ser Gly
50 55 60

Gln Glu Tyr Leu His Gln Ser Ala Asp Asn Asn Ile Val Leu Tyr Asn
65 70 75 80

Ile Glu Thr Gly Gln Ser Tyr Thr Ile Leu Ser Asn Arg Thr Met Lys
85 90 95

Ser Val Asn Ala Ser Asn Tyr Gly Leu Ser Pro Asp Arg Gln Phe Val
100 105 110

Tyr Leu Glu Ser Asp Tyr Ser Lys Leu Trp Arg Tyr Ser Tyr Thr Ala
115 120 125

Thr Tyr Tyr Ile Tyr Asp Leu Ser Asn Gly Glu Phe Val Arg Gly Asn
130 135 140

Glu Leu Pro Arg Pro Ile Gln Tyr Leu Cys Trp Ser Pro Val Gly Ser
145 150 155 160

Lys Leu Ala Tyr Val Tyr Gln Asn Asn Ile Tyr Leu Lys Gln Arg Pro
165 170 175

Gly Asp Pro Pro Phe Gln Ile Thr Phe Asn Gly Arg Glu Asn Lys Ile
180 185 190

Phe Asn Gly Ile Pro Asp Trp Val Tyr Glu Glu Glu Met Leu Ala Thr
195 200 205

Lys Tyr Ala Leu Trp Trp Ser Pro Asn Gly Lys Phe Leu Ala Tyr Ala
210 215 220

```
Glu Phe Asn Asp Thr Asp Ile Pro Val Ile Ala Tyr Ser Tyr Tyr Gly
225                 230                 235                 240

Asp Glu Gln Tyr Pro Arg Thr Ile Asn Ile Pro Tyr Pro Lys Ala Gly
                245                 250                 255

Ala Lys Asn Pro Val Val Arg Ile Phe Ile Ile Asp Thr Thr Tyr Pro
            260                 265                 270

Ala Tyr Val Gly Pro Gln Glu Val Pro Val Pro Ala Met Ile Ala Ser
        275                 280                 285

Ser Asp Tyr Tyr Phe Ser Trp Leu Thr Trp Val Thr Asp Glu Arg Val
    290                 295                 300

Cys Leu Gln Trp Leu Lys Arg Val Gln Asn Val Ser Val Leu Ser Ile
305                 310                 315                 320

Cys Asp Phe Arg Glu Asp Trp Gln Thr Trp Asp Cys Pro Lys Thr Gln
                325                 330                 335

Glu His Ile Glu Glu Ser Arg Thr Gly Trp Ala Gly Gly Phe Phe Val
            340                 345                 350

Ser Thr Pro Val Phe Ser Tyr Asp Ala Ile Ser Tyr Tyr Lys Ile Phe
        355                 360                 365

Ser Asp Lys Asp Gly Tyr Lys His Ile His Tyr Ile Lys Asp Thr Val
    370                 375                 380

Glu Asn Ala Ile Gln Ile Thr Ser Gly Lys Trp Glu Ala Ile Asn Ile
385                 390                 395                 400

Phe Arg Val Thr Gln Asp Ser Leu Phe Tyr Ser Ser Asn Glu Phe Glu
                405                 410                 415

Glu Tyr Pro Gly Arg Arg Asn Ile Tyr Arg Ile Ser Ile Gly Ser Tyr
            420                 425                 430

Pro Pro Ser Lys Lys Cys Val Thr Cys His Leu Arg Lys Glu Arg Cys
        435                 440                 445

Gln Tyr Tyr Thr Ala Ser Phe Ser Asp Tyr Ala Lys Tyr Tyr Ala Leu
    450                 455                 460

Val Cys Tyr Gly Pro Gly Ile Pro Ile Ser Thr Leu His Asp Gly Arg
465                 470                 475                 480
```

Thr Asp Gln Glu Ile Lys Ile Leu Glu Glu Asn Lys Glu Leu Glu Asn
485 490 495

Ala Leu Lys Asn Ile Gln Leu Pro Lys Glu Glu Ile Lys Lys Leu Glu
500 505 510

Val Asp Glu Ile Thr Leu Trp Tyr Lys Met Ile Leu Pro Pro Gln Phe
515 520 525

Asp Arg Ser Lys Lys Tyr Pro Leu Leu Ile Gln Val Tyr Gly Gly Pro
530 535 540

Cys Ser Gln Ser Val Arg Ser Val Phe Ala Val Asn Trp Ile Ser Tyr
545 550 555 560

Leu Ala Ser Lys Glu Gly Met Val Ile Ala Leu Val Asp Gly Arg Gly
565 570 575

Thr Ala Phe Gln Gly Asp Lys Leu Leu Tyr Ala Val Tyr Arg Lys Leu
580 585 590

Gly Val Tyr Glu Val Glu Asp Gln Ile Thr Ala Val Arg Lys Phe Ile
595 600 605

Glu Met Gly Phe Ile Asp Glu Lys Arg Ile Ala Ile Trp Gly Trp Ser
610 615 620

Tyr Gly Gly Tyr Val Ser Ser Leu Ala Leu Ala Ser Gly Thr Gly Leu
625 630 635 640

Phe Lys Cys Gly Ile Ala Val Ala Pro Val Ser Ser Trp Glu Tyr Tyr
645 650 655

Ala Ser Val Tyr Thr Glu Arg Phe Met Gly Leu Pro Thr Lys Asp Asp
660 665 670

Asn Leu Glu His Tyr Lys Asn Ser Thr Val Met Ala Arg Ala Glu Tyr
675 680 685

Phe Arg Asn Val Asp Tyr Leu Leu Ile His Gly Thr Ala Asp Asp Asn
690 695 700

Val His Phe Gln Asn Ser Ala Gln Ile Ala Lys Ala Leu Val Asn Ala
705 710 715 720

Gln Val Asp Phe Gln Ala Met Trp Tyr Ser Asp Gln Asn His Gly Leu
725 730 735

Ser Gly Leu Ser Thr Asn His Leu Tyr Thr His Met Thr His Phe Leu
740 745 750

Lys Gln Cys Phe Ser Leu Ser Asp
755 760

<210> 3
<211> 2255
<212> DNA
<213> Homo sapiens

<400> 3
gacgcggccc agccggccac aaagagagct cttaccctga aggatatttt aaatggaaca 60

ttttcttata aaacattttt tccaaactgg atttcaggac aagaatatct tcatcaatct 120

gcagataaca atatagtact ttataatatt gaaacaggac aatcatatac cattttgagt 180

aatagaacca tgaaaagtgt gaatgcttca aattacggct tatcacctga tcggcaattt 240

gtatatctag aaagtgatta ttcaaagctt tggagatact cttacacagc aacatattac 300

atctatgacc ttagcaatgg agaatttgta agaggaaatg agcttcctcg tccaattcag 360

tatttatgct ggtcgcctgt tgggagtaaa ttagcatatg tctatcaaaa caatatctat 420

ttgaaacaaa gaccaggaga tccacctttt caaataacat ttaatggaag agaaaataaa 480

atatttaatg gaatcccaga ctgggtttat gaagaggaaa tgcttgctac aaaatatgct 540

ctctggtggt ctcctaatgg aaaatttttg gcatatgcgg aatttaatga tatggatata 600

ccagttattg cctattccta ttatggcgat gaacaatatc ctagaacaat aaatattcca 660

tacccaaagg ctggagctaa gaatcccgtt gttcggatat ttattatcga taccacttac 720

cctgcgtatg taggtcccca ggaagtgcct gttccagcaa tgatagcctc aagtgattat 780

tatttcagtt ggctcacgtg ggttactgat gaacgagtat gtttgcagtg gctaaaaaga 840

gtccagaatg tttcggtcct gtctatatgt gacttcaggg aagactggca gacatgggat 900

tgtccaaaga cccaggagca tatagaagaa agcagaactg gatgggctgg tggattcttt 960

gtttcaacac cagttttcag ctatgatgcc atttcgtact acaaaatatt tagtgacaag 1020

gatggctaca aacatattca ctatatcaaa gacactgtgg aaaatgctat tcaaattaca 1080

agtggcaagt gggaggccat aaatatattc agagtaacac aggattcact gttttattct 1140

agcaatgaat ttgaagaata ccctggaaga agaaacatct acagaattag cattggaagc 1200

tatcctccaa gcaagaagtg tgttacttgc catctaagga aagaaaggtg ccaatattac 1260

acagcaagtt tcagcgacta cgccaagtac tatgcacttg tctgctacgg cccaggcatc 1320

cccatttcca cccttcatga tggacgcact gatcaagaaa ttaaaatcct ggaagaaaac 1380

aaggaattgg aaaatgcttt gaaaaatatc cagctgccta aagaggaaat taagaaactt 1440

gaagtagatg aaattacttt atggtacaag atgattcttc ctcctcaatt tgacagatca 1500

aagaagtatc ccttgctaat tcaagtgtat ggtggtccct gcagtcagag tgtaaggtct 1560

gtatttgctg ttaattggat atcttatctt gcaagtaagg aagggatggt cattgccttg 1620

gtggatggtc gaggaacagc tttccaaggt gacaaactcc tctatgcagt gtatcgaaag 1680

ctgggtgttt atgaagttga agaccagatt acagctgtca gaaaattcat agaaatgggt 1740

ttcattgatg aaaaaagaat agccatatgg ggctggtcct atggaggata cgtttcatca 1800

ctggcccttg catctggaac tggtcttttc aaatgtggta tagcagtggc tccagtctcc 1860

agctgggaat attacgcgtc tgtctacaca gagagattca tgggtctccc aacaaaggat 1920

gataatcttg agcactataa gaattcaact gtgatggcaa gagcagaata tttcagaaat 1980

gtagactatc ttctcatcca cggaacagca gatgataatg tgcactttca aaactcagca 2040

cagattgcta aagctctggt taatgcacaa gtggatttcc aggcaatgtg gtactctgac 2100

cagaaccacg gcttatccgg cctgtccacg aaccacttat acacccacat gacccacttc 2160

ctaaagcagt gtttctcttt gtcagactaa aaacgatgca gatgcaagcc tgtatcagaa 2220

tctgaaaggg cgaattccag cacagtggcg gccgc 2255

<210> 4
<211> 750
<212> PRT
<213> Homo sapiens

<400> 4

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1 5 10 15

Gly Ser Thr Gly Asp Ala Ala Gln Pro Ala Thr Lys Arg Ala Leu Thr
20 25 30

Leu Lys Asp Ile Leu Asn Gly Thr Phe Ser Tyr Lys Thr Phe Phe Pro
35 40 45

Asn Trp Ile Ser Gly Gln Glu Tyr Leu His Gln Ser Ala Asp Asn Asn
50 55 60

Ile Val Leu Tyr Asn Ile Glu Thr Gly Gln Ser Tyr Thr Ile Leu Ser
65 70 75 80

Asn Arg Thr Met Lys Ser Val Asn Ala Ser Asn Tyr Gly Leu Ser Pro
85 90 95

```
Asp Arg Gln Phe Val Tyr Leu Glu Ser Asp Tyr Ser Lys Leu Trp Arg
            100                 105                 110

Tyr Ser Tyr Thr Ala Thr Tyr Tyr Ile Tyr Asp Leu Ser Asn Gly Glu
        115                 120                 125

Phe Val Arg Gly Asn Glu Leu Pro Arg Pro Ile Gln Tyr Leu Cys Trp
    130                 135                 140

Ser Pro Val Gly Ser Lys Leu Ala Tyr Val Tyr Gln Asn Asn Ile Tyr
145                 150                 155                 160

Leu Lys Gln Arg Pro Gly Asp Pro Pro Phe Gln Ile Thr Phe Asn Gly
                165                 170                 175

Arg Glu Asn Lys Ile Phe Asn Gly Ile Pro Asp Trp Val Tyr Glu Glu
            180                 185                 190

Glu Met Leu Ala Thr Lys Tyr Ala Leu Trp Trp Ser Pro Asn Gly Lys
        195                 200                 205

Phe Leu Ala Tyr Ala Glu Phe Asn Asp Met Asp Ile Pro Val Ile Ala
    210                 215                 220

Tyr Ser Tyr Tyr Gly Asp Glu Gln Tyr Pro Arg Thr Ile Asn Ile Pro
225                 230                 235                 240

Tyr Pro Lys Ala Gly Ala Lys Asn Pro Val Val Arg Ile Phe Ile Ile
                245                 250                 255

Asp Thr Thr Tyr Pro Ala Tyr Val Gly Pro Gln Glu Val Pro Val Pro
            260                 265                 270

Ala Met Ile Ala Ser Ser Asp Tyr Tyr Phe Ser Trp Leu Thr Trp Val
        275                 280                 285

Thr Asp Glu Arg Val Cys Leu Gln Trp Leu Lys Arg Val Gln Asn Val
    290                 295                 300

Ser Val Leu Ser Ile Cys Asp Phe Arg Glu Asp Trp Gln Thr Trp Asp
305                 310                 315                 320

Cys Pro Lys Thr Gln Glu His Ile Glu Glu Ser Arg Thr Gly Trp Ala
                325                 330                 335
```

```
Gly Gly Phe Phe Val Ser Thr Pro Val Phe Ser Tyr Asp Ala Ile Ser
            340                 345                 350

Tyr Tyr Lys Ile Phe Ser Asp Lys Asp Gly Tyr Lys His Ile His Tyr
        355                 360                 365

Ile Lys Asp Thr Val Glu Asn Ala Ile Gln Ile Thr Ser Gly Lys Trp
    370                 375                 380

Glu Ala Ile Asn Ile Phe Arg Val Thr Gln Asp Ser Leu Phe Tyr Ser
385                 390                 395                 400

Ser Asn Glu Phe Glu Glu Tyr Pro Gly Arg Arg Asn Ile Tyr Arg Ile
                405                 410                 415

Ser Ile Gly Ser Tyr Pro Pro Ser Lys Lys Cys Val Thr Cys His Leu
            420                 425                 430

Arg Lys Glu Arg Cys Gln Tyr Tyr Thr Ala Ser Phe Ser Asp Tyr Ala
        435                 440                 445

Lys Tyr Tyr Ala Leu Val Cys Tyr Gly Pro Gly Ile Pro Ile Ser Thr
    450                 455                 460

Leu His Asp Gly Arg Thr Asp Gln Glu Ile Lys Ile Leu Glu Glu Asn
465                 470                 475                 480

Lys Glu Leu Glu Asn Ala Leu Lys Asn Ile Gln Leu Pro Lys Glu Glu
                485                 490                 495

Ile Lys Lys Leu Glu Val Asp Glu Ile Thr Leu Trp Tyr Lys Met Ile
            500                 505                 510

Leu Pro Pro Gln Phe Asp Arg Ser Lys Lys Tyr Pro Leu Leu Ile Gln
        515                 520                 525

Val Tyr Gly Gly Pro Cys Ser Gln Ser Val Arg Ser Val Phe Ala Val
    530                 535                 540

Asn Trp Ile Ser Tyr Leu Ala Ser Lys Glu Gly Met Val Ile Ala Leu
545                 550                 555                 560

Val Asp Gly Arg Gly Thr Ala Phe Gln Gly Asp Lys Leu Leu Tyr Ala
                565                 570                 575

Val Tyr Arg Lys Leu Gly Val Tyr Glu Val Glu Asp Gln Ile Thr Ala
            580                 585                 590
```

Val Arg Lys Phe Ile Glu Met Gly Phe Ile Asp Glu Lys Arg Ile Ala
595 600 605

Ile Trp Gly Trp Ser Tyr Gly Gly Tyr Val Ser Ser Leu Ala Leu Ala
610 615 620

Ser Gly Thr Gly Leu Phe Lys Cys Gly Ile Ala Val Ala Pro Val Ser
625 630 635 640

Ser Trp Glu Tyr Tyr Ala Ser Val Tyr Thr Glu Arg Phe Met Gly Leu
645 650 655

Pro Thr Lys Asp Asp Asn Leu Glu His Tyr Lys Asn Ser Thr Val Met
660 665 670

Ala Arg Ala Glu Tyr Phe Arg Asn Val Asp Tyr Leu Leu Ile His Gly
675 680 685

Thr Ala Asp Asp Asn Val His Phe Gln Asn Ser Ala Gln Ile Ala Lys
690 695 700

Ala Leu Val Asn Ala Gln Val Asp Phe Gln Ala Met Trp Tyr Ser Asp
705 710 715 720

Gln Asn His Gly Leu Ser Gly Leu Ser Thr Asn His Leu Tyr Thr His
725 730 735

Met Thr His Phe Leu Lys Gln Cys Phe Ser Leu Ser Asp Asn
740 745 750

<210> 5
<211> 2715
<212> DNA
<213> Mus musculus

<400> 5
acggtagtca aaagtccgtg ggaaagggag gaggggagcg ggaagcaact cctgtcctga 60

cttcggcttc caagtggagg acagacttgc ttcttttcaa cagttttcac agatgcggtg 120

acccacgctg tgcagtgaga atcagctaac ttgcaaaaac atctggaaaa atgaagacat 180

ggctgaaaac tgtctttgga gttaccaccc tggctgcgct tgctttagtg gtgatatgca 240

ttgtcttacg tccctcaaga gtttacaaac ctgaaggaaa cacaaagaga gctcttacct 300

tgaaggatat tttaaatgga acattctcat ataaaacata ttttcccaac tggatttcag 360

aacaagaata tcttcatcaa tctgaggatg ataacatagt attttataat attgaaacaa 420

gagaatcata tatcattttg agtaatagca ccatgaaaag tgtgaatgct acagattatg 480

gtttgtcacc tgatcggcaa tttgtgtatc tagaaagtga ttattcaaag ctctggcgat 540

attcatacac agcgacatac tacatctacg accttcagaa tggggaattt gtaagaggat 600

acgagctccc tcgtccaatt cagtatctat gctggtcgcc tgttgggagt aaattagcat 660

atgtatatca aaacaatatt tatttgaaac aaagaccagg agatccacct tttcaaataa 720

cttatactgg aagagaaaat agaatattta atggaatacc agactgggtt tatgaagagg 780

aaatgcttgc cacaaaatat gctctttggt ggtctccaga tggaaaattt ttggcatatg 840

tagaatttaa tgattcagat ataccaatta ttgcctattc ttattatggt gatggacagt 900

atcctagaac tataaatatt ccatatccaa aggctggggc taagaatccg gttgttcgtg 960

tttttattgt tgacaccacc taccctcacc acgtgggccc aatggaagtg ccagttccag 1020

aaatgatagc ctcaagtgac tattatttca gctggctcac atgggtgtcc agtgaacgag 1080

tatgcttgca gtggctaaaa agagtgcaga atgtctcagt cctgtctata tgtgatttca 1140

gggaagactg gcatgcatgg gaatgtccaa agaaccagga gcatgtagaa gaaagcagaa 1200

caggatgggc tggtggattc tttgtttcga caccagcttt tagccaggat gccacttctt 1260

actacaaaat atttagcgac aaggatggtt acaaacatat tcactacatc aaagacactg 1320

tggaaaatgc tattcaaatt acaagtggca agtgggaggc catatatata ttccgcgtaa 1380

cacaggattc actgttttat tctagcaatg aatttgaagg ttaccctgga agaagaaaca 1440

tctacagaat tagcattgga aactctcctc cgagcaagaa gtgtgttact tgccatctaa 1500

ggaaagaaag gtgccaatat tacacagcaa gtttcagcta caaagccaag tactatgcac 1560

tcgtctgcta tggccctggc ctccccattt ccaccctcca tgatggccgc acagaccaag 1620

aaatacaagt attagaagaa aacaaagaac tggaaaattc tctgagaaat atccagctgc 1680

ctaaagtgga gattaagaag ctcaaagacg ggggactgac tttctggtac aagatgattc 1740

tgcctcctca gtttgacaga tcaaagaagt acccttttgct aattcaagtg tatggtggtc 1800

cttgtagcca gagtgttaag tctgtgtttg ctgttaattg gataacttat ctcgcaagta 1860

aggaggggat agtcattgcc ctggtagatg gtcggggcac tgctttccaa ggtgacaaat 1920

tcctgcatgc cgtgtatcga aaactgggtg tatatgaagt tgaggaccag ctcacagctg 1980

tcagaaaatt catagaaatg ggtttcattg atgaagaaag aatagccata tggggctggt 2040

cctacggagg ttatgtttca tccctggccc ttgcatctgg aactggtctt ttcaaatgtg 2100

gcatagcagt ggctccagtc tccagctggg aatattacgc atctatctac tcagagagat 2160

tcatgggcct cccaacaaag gacgacaatc tcgaacacta taaaaattca actgtgatgg 2220

caagagcaga atatttcaga aatgtagact atcttctcat ccacggaaca gcagatgata 2280

atgtgcactt tcagaactca gcacagattg ctaaagcttt ggttaatgca caagtggatt 2340

tccaggcgat gtggtactct gaccagaacc atggtatatc atctgggcgc tcccagaatc 2400

atttatatac ccacatgacg cacttcctca agcaatgctt ttctttatca gactgaacca 2460

atgcaagtac tagcgtgtag gacagtctga aggcttcaca tggacgactt ggaccatgaa 2520

tattgtaaaa gccgctaaaa atttaggagg gctcaggagg ttaggctcgg tattgtttac 2580

atttccctat gctctgtgaa agaagagaaa aggggagcca cacatcttgc tttggacaca 2640

atgttttatc acctgttcat tgccgggtta gcggaaataa agtcagatgc tcaggtaaaa 2700

aaaaaaaaaa aaaaa 2715

<210> 6
<211> 761
<212> PRT
<213> Mus musculus

<400> 6

Met Lys Thr Trp Leu Lys Thr Val Phe Gly Val Thr Thr Leu Ala Ala
1 5 10 15

Leu Ala Leu Val Val Ile Cys Ile Val Leu Arg Pro Ser Arg Val Tyr
20 25 30

Lys Pro Glu Gly Asn Thr Lys Arg Ala Leu Thr Leu Lys Asp Ile Leu
35 40 45

Asn Gly Thr Phe Ser Tyr Lys Thr Tyr Phe Pro Asn Trp Ile Ser Glu
50 55 60

Gln Glu Tyr Leu His Gln Ser Glu Asp Asp Asn Ile Val Phe Tyr Asn
65 70 75 80

Ile Glu Thr Arg Glu Ser Tyr Ile Ile Leu Ser Asn Ser Thr Met Lys
85 90 95

Ser Val Asn Ala Thr Asp Tyr Gly Leu Ser Pro Asp Arg Gln Phe Val
100 105 110

Tyr Leu Glu Ser Asp Tyr Ser Lys Leu Trp Arg Tyr Ser Tyr Thr Ala
115 120 125

Thr Tyr Tyr Ile Tyr Asp Leu Gln Asn Gly Glu Phe Val Arg Gly Tyr
130 135 140

Glu Leu Pro Arg Pro Ile Gln Tyr Leu Cys Trp Ser Pro Val Gly Ser
145 150 155 160

```
Lys Leu Ala Tyr Val Tyr Gln Asn Asn Ile Tyr Leu Lys Gln Arg Pro
                165                 170                 175

Gly Asp Pro Pro Phe Gln Ile Thr Tyr Thr Gly Arg Glu Asn Arg Ile
            180                 185                 190

Phe Asn Gly Ile Pro Asp Trp Val Tyr Glu Glu Glu Met Leu Ala Thr
        195                 200                 205

Lys Tyr Ala Leu Trp Trp Ser Pro Asp Gly Lys Phe Leu Ala Tyr Val
    210                 215                 220

Glu Phe Asn Asp Ser Asp Ile Pro Ile Ile Ala Tyr Ser Tyr Tyr Gly
225                 230                 235                 240

Asp Gly Gln Tyr Pro Arg Thr Ile Asn Ile Pro Tyr Pro Lys Ala Gly
                245                 250                 255

Ala Lys Asn Pro Val Val Arg Val Phe Ile Val Asp Thr Thr Tyr Pro
            260                 265                 270

His His Val Gly Pro Met Glu Val Pro Val Pro Glu Met Ile Ala Ser
        275                 280                 285

Ser Asp Tyr Tyr Phe Ser Trp Leu Thr Trp Val Ser Ser Glu Arg Val
    290                 295                 300

Cys Leu Gln Trp Leu Lys Arg Val Gln Asn Val Ser Val Leu Ser Ile
305                 310                 315                 320

Cys Asp Phe Arg Glu Asp Trp His Ala Trp Glu Cys Pro Lys Asn Gln
                325                 330                 335

Glu His Val Glu Glu Ser Arg Thr Gly Trp Ala Gly Gly Phe Phe Val
            340                 345                 350

Ser Thr Pro Ala Phe Ser Gln Asp Ala Thr Ser Tyr Tyr Lys Ile Phe
        355                 360                 365

Ser Asp Lys Asp Gly Tyr Lys His Ile His Tyr Ile Lys Asp Thr Val
    370                 375                 380

Glu Asn Ala Ile Gln Ile Thr Ser Gly Lys Trp Glu Ala Ile Tyr Ile
385                 390                 395                 400
```

```
Phe Arg Val Thr Gln Asp Ser Leu Phe Tyr Ser Ser Asn Glu Phe Glu
                405                 410                 415

Gly Tyr Pro Gly Arg Arg Asn Ile Tyr Arg Ile Ser Ile Gly Asn Ser
            420                 425                 430

Pro Pro Ser Lys Lys Cys Val Thr Cys His Leu Arg Lys Glu Arg Cys
        435                 440                 445

Gln Tyr Tyr Thr Ala Ser Phe Ser Tyr Lys Ala Lys Tyr Tyr Ala Leu
    450                 455                 460

Val Cys Tyr Gly Pro Gly Leu Pro Ile Ser Thr Leu His Asp Gly Arg
465                 470                 475                 480

Thr Asp Gln Glu Ile Gln Val Leu Glu Glu Asn Lys Glu Leu Glu Asn
                485                 490                 495

Ser Leu Arg Asn Ile Gln Leu Pro Lys Val Glu Ile Lys Lys Leu Lys
            500                 505                 510

Asp Gly Gly Leu Thr Phe Trp Tyr Lys Met Ile Leu Pro Pro Gln Phe
        515                 520                 525

Asp Arg Ser Lys Lys Tyr Pro Leu Leu Ile Gln Val Tyr Gly Gly Pro
    530                 535                 540

Cys Ser Gln Ser Val Lys Ser Val Phe Ala Val Asn Trp Ile Thr Tyr
545                 550                 555                 560

Leu Ala Ser Lys Glu Gly Ile Val Ile Ala Leu Val Asp Gly Arg Gly
                565                 570                 575

Thr Ala Phe Gln Gly Asp Lys Phe Leu His Ala Val Tyr Arg Lys Leu
            580                 585                 590

Gly Val Tyr Glu Val Glu Asp Gln Leu Thr Ala Val Arg Lys Phe Ile
        595                 600                 605

Glu Met Gly Phe Ile Asp Glu Glu Arg Ile Ala Ile Trp Gly Trp Ser
    610                 615                 620

Tyr Gly Gly Tyr Val Ser Ser Leu Ala Leu Ala Ser Gly Thr Gly Leu
625                 630                 635                 640

Phe Lys Cys Gly Ile Ala Val Ala Pro Val Ser Ser Trp Glu Tyr Tyr
                645                 650                 655
```

Ala Ser Ile Tyr Ser Glu Arg Phe Met Gly Leu Pro Thr Lys Asp Asp
660 665 670

Asn Leu Glu His Tyr Lys Asn Ser Thr Val Met Ala Arg Ala Glu Tyr
675 680 685

Phe Arg Asn Val Asp Tyr Leu Leu Ile His Gly Thr Ala Asp Asp Asn
690 695 700

Val His Phe Gln Asn Ser Ala Gln Ile Ala Lys Ala Leu Val Asn Ala
705 710 715 720

Gln Val Asp Phe Gln Ala Met Trp Tyr Ser Asp Gln Asn His Gly Ile
725 730 735

Ser Ser Gly Arg Ser Gln Asn His Leu Tyr Thr His Met Thr His Phe
740 745 750

Leu Lys Gln Cys Phe Ser Leu Ser Asp
755 760

<210> 7
<211> 33
<212> PRT
<213> Homo sapiens

<400> 7

Thr Glu Val Pro Leu Ile Glu Tyr Ser Phe Tyr Ser Asp Glu Ser Leu
1 5 10 15

Gln Tyr Pro Lys Thr Val Arg Val Pro Tyr Pro Lys Ala Gly Ala Val
20 25 30

Asn

<210> 8
<211> 31
<212> PRT
<213> Homo sapiens

<400> 8

Thr Asp Ile Pro Val Ile Ala Tyr Ser Tyr Tyr Gly Asp Glu Gln Tyr
1 5 10 15

Pro Arg Thr Ile Asn Ile Pro Tyr Pro Lys Ala Gly Ala Lys Asn
20 25 30

<210> 9
<211> 68
<212> PRT
<213> Homo sapiens

<400> 9

Val Glu Tyr Leu Leu Ile His Gly Thr Ala Asp Asp Asn Val His Phe
1 5 10 15

Gln Gln Ser Ala Gln Ile Ser Lys Ala Leu Val Asp Val Gly Val Asp
20 25 30

Phe Gln Ala Met Trp Tyr Thr Asp Glu Asp His Gly Ile Ala Ser Ser
35 40 45

Thr Ala His Gln His Ile Tyr Thr His Met Ser His Phe Ile Lys Gln
50 55 60

Cys Phe Ser Leu
65

<210> 10
<211> 67
<212> PRT
<213> Homo sapiens

<400> 10

Val Asp Tyr Leu Leu Ile His Gly Thr Ala Asp Asp Asn Val His Phe
1 5 10 15

Gln Asn Ser Ala Gln Ile Ala Lys Ala Leu Val Asn Ala Gln Val Asp
20 25 30

Phe Gln Ala Met Trp Tyr Ser Asp Gln Asn His Gly Leu Ser Gly Leu
35 40 45

Ser Thr Asn His Leu Tyr Thr His Met Thr His Phe Leu Lys Gln Cys
50 55 60

Phe Ser Leu
65

<210> 11
<211> 24
<212> DNA
<213> artificial sequence

<220>

<223> PCR primer

<400> 11
ccacgctctg aagacagaat tagc 24

<210> 12
<211> 22
<212> DNA
<213> artificial sequence

<220>
<223> PCR primer

<400> 12
tcagattctg atagaggctt gc 22

<210> 13
<211> 56
<212> DNA
<213> artificial sequence

<220>
<223> PCR primer

<400> 13
gtagtcggcc cagccggcca caaagagagc tcttaccctg aaggatattt taaatg 56

<210> 14
<211> 6
<212> PRT
<213> artificial sequence

<220>
<223> vector derived sequence

<400> 14

Asp Ala Ala Gln Pro Ala
1 5

<210> 15
<211> 13
<212> PRT
<213> artificial sequence

<220>
<223> vector derived sequence

<400> 15

Thr Lys Arg Ala Leu Thr Leu Lys Asp Ile Leu Asn Gly
1 5 10

<210> 16
<211> 51
<212> PRT
<213> Homo sapiens

<400> 16

Met Lys Thr Pro Trp Lys Val Leu Leu Gly Leu Leu Gly Ala Ala Ala
1 5 10 15

Leu Val Thr Ile Ile Thr Val Pro Val Val Leu Leu Asn Lys Gly Thr
20 25 30

Asp Asp Ala Thr Ala Asp Ser Arg Lys Thr Tyr Thr Leu Thr Asp Tyr
35 40 45

Leu Lys Asn
50

<210> 17
<211> 13
<212> PRT
<213> Homo sapiens

<400> 17

Ser Arg Lys Thr Tyr Thr Leu Thr Asp Tyr Leu Lys Asn
1 5 10

<210> 18
<211> 12
<212> PRT
<213> Homo sapiens

<400> 18

Arg Lys Thr Tyr Thr Leu Thr Asp Tyr Leu Lys Asn
1 5 10

<210> 19
<211> 50
<212> PRT
<213> Homo sapiens

<400> 19

Met Lys Thr Trp Val Lys Ile Val Phe Gly Val Ala Thr Ser Ala Val
1 5 10 15

Leu Ala Leu Leu Val Met Cys Ile Val Leu Arg Pro Ser Arg Val His
20 25 30

Asn Ser Glu Glu Asn Thr Met Arg Ala Leu Thr Leu Lys Asp Ile Leu
35 40 45

Asn Gly
50

<210> 20
<211> 13
<212> PRT
<213> Homo sapiens

<400> 20

Thr Met Arg Ala Leu Thr Leu Lys Asp Ile Leu Asn Gly
1 5 10

<210> 21
<211> 50
<212> PRT
<213> Mus musculus

<400> 21

Met Lys Thr Trp Leu Lys Thr Val Phe Gly Val Thr Thr Leu Ala Ala
1 5 10 15

Leu Ala Leu Val Val Ile Cys Ile Val Leu Arg Pro Ser Arg Val Tyr
20 25 30

Lys Pro Glu Gly Asn Thr Lys Arg Ala Leu Thr Leu Lys Asp Ile Leu
35 40 45

Asn Gly
50

<210> 22
<211> 13
<212> PRT
<213> Mus musculus

<400> 22

Thr Lys Arg Ala Leu Thr Leu Lys Asp Ile Leu Asn Gly
1 5 10

<210> 23
<211> 51
<212> DNA
<213> artificial sequence

<220>
<223> PCR primer

<400> 23
gtactgctgc tctgggttcc aggttccact ggtgacgcgg cccagccggc c 51

<210> 24
<211> 17
<212> PRT

<213> artificial sequence

<220>
<223> PCR primer

<400> 24

Val Leu Leu Leu Trp Val Pro Gly Ser Thr Gly Asp Ala Ala Gln Pro
1 5 10 15

Ala

<210> 25
<211> 20
<212> PRT
<213> artificial sequence

<220>
<223> PCR primer

<400> 25

Gly Asp Ala Ala Gln Pro Ala Thr Lys Arg Ala Leu Thr Leu Lys Asp
1 5 10 15

Ile Leu Asn Gly
20

<210> 26
<211> 41
<212> DNA
<213> artificial sequence

<220>
<223> PCR primer

<400> 26
ccaagctggc tagccaccat ggctggacct gccacccaga g 41

<210> 27
<211> 27
<212> DNA
<213> artificial sequence

<220>
<223> PCR primer

<400> 27
ggcttcctgc actgtccaga gtgcact 27

<210> 28
<211> 57
<212> DNA
<213> artificial sequence

<220>
<223> PCR primer

<400> 28
gcactctgga cagtgcagga agccacaaag agagctctta ccctgaagga tatttta 57

<210> 29
<211> 32
<212> DNA
<213> artificial sequence

<220>
<223> PCR primer

<400> 29
gcagggtaag tggtatcgat aataaatatc cg 32

<210> 30
<211> 58
<212> DNA
<213> artificial sequence

<220>
<223> PCR primer

<400> 30
gtagtcggcc cagccggcca gtcgcaaaac ttacactcta actgattact taaaaaat 58

<210> 31
<211> 47
<212> DNA
<213> artificial sequence

<220>
<223> PCR primer

<400> 31
gtcggagcgg ccgcctaagg taaagagaaa cattgtttta tgaagtg 47

<210> 32
<211> 41
<212> DNA
<213> artificial sequence

<220>
<223> PCR primer

<400> 32
tccagctggg aatattacga ctctgtctac acagagagat t 41

<210> 33
<211> 41
<212> DNA
<213> artificial sequence

<220>
<223> PCR primer

<400> 33
aatctctctg tgtagacaga gtcgtaatat tcccagctgg a 41

<210> 34
<211> 37
<212> DNA
<213> artificial sequence

<220>
<223> PCR primer

<400> 34
tagatggaaa ttacttatgg tacaagatga ttcttcc 37

<210> 35
<211> 47
<212> DNA
<213> artificial sequence

<220>
<223> PCR primer

<400> 35
ggtcgctcag cggccgctta gtctgacaaa gagaaacact gctttag 47

<210> 36
<211> 52
<212> DNA
<213> artificial sequence

<220>
<223> PCR primer

<400> 36
tttgtatatc tagaaagtga ttattcaaag ctttggagac actcttacac ag 52

<210> 37
<211> 36
<212> DNA
<213> artificial sequence

<220>
<223> PCR primer

<400> 37
ccagagagca tattttgtag aaagcatttc ctcttc 36

<210> 38
<211> 36
<212> DNA
<213> artificial sequence

<220>
<223> PCR primer

<400> 38

gaagaggaaa tgctttctac aaaatatgct ctctgg 36

<210> 39
<211> 32
<212> DNA
<213> artificial sequence

<220>
<223> PCR primer

<400> 39
cggatattta ttatcgatac cacttaccct gc 32

<210> 40
<211> 52
<212> DNA
<213> artificial sequence

<220>
<223> PCR primer

<400> 40
tgaaggccta aatcttccaa cccatccagt tctgctttct tctatatgct cc 52

<210> 41
<211> 46
<212> DNA
<213> artificial sequence

<220>
<223> PCR primer

<400> 41
tgggttggaa gatttaggcc ttcaacacca gttttcagct atgatg 46

<210> 42
<211> 50
<212> DNA
<213> artificial sequence

<220>
<223> PCR primer

<400> 42
ctgtatttgc tgttaattgg atatcttacc ttgcaagcac agaaaacatt 50

<210> 43
<211> 37
<212> DNA
<213> artificial sequence

<220>
<223> PCR primer

<400> 43
tagatggaaa ttacttatgg tacaagatga ttcttcc 37

<210> 44
<211> 41
<212> DNA
<213> artificial sequence

<220>
<223> PCR primer

<400> 44
aatgtggtac tctgacgaag accacggctt atccggcctg t 41

<210> 45
<211> 30
<212> DNA
<213> artificial sequence

<220>
<223> PCR primer

<400> 45
tggtcttcgt cagagtacca cattgcctgg 30

<210> 46
<211> 27
<212> DNA
<213> artificial sequence

<220>
<223> PCR primer

<400> 46
ggcgctattc agatcctctt ctgagat 27

<210> 47
<211> 48
<212> DNA
<213> artificial sequence

<220>
<223> PCR primer

<400> 47
ggatgataat cttgagcact ataagaattc aacagtcatg agcagagc 48

<210> 48
<211> 46
<212> DNA
<213> artificial sequence

<220>
<223> PCR primer

<400> 48
aggccggata agccatggtc ttcatcagta taccacattg cctgga 46

<210> 49

<211> 46
<212> DNA
<213> artificial sequence

<220>
<223> PCR primer

<400> 49
caatgtggta tactgatgaa gaccatggct tatccggcct gtccac 46

<210> 50
<211> 35
<212> DNA
<213> artificial sequence

<220>
<223> PCR primer

<400> 50
tcccggtggg agtactatgc ctcagtgtac acaga 35

<210> 51
<211> 35
<212> DNA
<213> artificial sequence

<220>
<223> PCR primer

<400> 51
tctgtgtaca ctgaggcata gtactcccac cggga 35

<210> 52
<211> 29
<212> DNA
<213> artificial sequence

<220>
<223> PCR primer

<400> 52
aagactgcac atttattaca aaaggcacc 29

<210> 53
<211> 42
<212> DNA
<213> artificial sequence

<220>
<223> PCR primer

<400> 53
gacatttatg atttaaataa aaggcagctg attacagaag ag 42

<210> 54
<211> 46
<212> DNA

<213> artificial sequence

<220>
<223> PCR primer

<400> 54
ctgaagcgaa aaaacctcca gcccagccag tagtactcat tcaatg 46

<210> 55
<211> 45
<212> DNA
<213> artificial sequence

<220>
<223> PCR primer

<400> 55
gctggaggtt ttttcgcttc agaacctcat tttacccttg atggt 45

<210> 56
<211> 30
<212> DNA
<213> artificial sequence

<220>
<223> PCR primer

<400> 56
tagtactgac acctttccgg attcagctca 30

<210> 57
<211> 54
<212> DNA
<213> artificial sequence

<220>
<223> PCR primer

<400> 57
gccttttatt taaatcataa atgtcatatg aagctgtgta ggaatacctc catt 54

<210> 58
<211> 32
<212> DNA
<213> artificial sequence

<220>
<223> PCR primer

<400> 58
accaccacag agcagcgtag gcactgaaga ct 32

<210> 59
<211> 32
<212> DNA
<213> artificial sequence

<220>
<223> PCR primer

<400> 59
agtcttcagt gcctactatg ctctgtggtg gt 32

<210> 60
<211> 21
<212> DNA
<213> artificial sequence

<220>
<223> PCR primer

<400> 60
ttccattggg cccacgtggt g 21

<210> 61
<211> 734
<212> PRT
<213> Homo Sapiens

<400> 61

Arg Pro Ser Arg Val His Asn Ser Glu Glu Asn Thr Met Arg Ala Leu
1 5 10 15

Thr Leu Lys Asp Ile Leu Asn Gly Thr Phe Ser Tyr Lys Thr Phe Phe
20 25 30

Pro Asn Trp Ile Ser Gly Gln Glu Tyr Leu His Gln Ser Ala Asp Asn
35 40 45

Asn Ile Val Leu Tyr Asn Ile Glu Thr Gly Gln Ser Tyr Thr Ile Leu
50 55 60

Ser Asn Arg Thr Met Lys Ser Val Asn Ala Ser Asn Tyr Gly Leu Ser
65 70 75 80

Pro Asp Arg Gln Phe Val Tyr Leu Glu Ser Asp Tyr Ser Lys Leu Trp
85 90 95

Arg Tyr Ser Tyr Thr Ala Thr Tyr Tyr Ile Tyr Asp Leu Ser Asn Gly
100 105 110

Glu Phe Val Arg Gly Asn Glu Leu Pro Arg Pro Ile Gln Tyr Leu Cys
115 120 125

Trp Ser Pro Val Gly Ser Lys Leu Ala Tyr Val Tyr Gln Asn Asn Ile
130 135 140

```
Tyr Leu Lys Gln Arg Pro Gly Asp Pro Pro Phe Gln Ile Thr Phe Asn
145                 150                 155                 160

Gly Arg Glu Asn Lys Ile Phe Asn Gly Ile Pro Asp Trp Val Tyr Glu
                165                 170                 175

Glu Glu Met Leu Ala Thr Lys Tyr Ala Leu Trp Trp Ser Pro Asn Gly
            180                 185                 190

Lys Phe Leu Ala Tyr Ala Glu Phe Asn Asp Thr Asp Ile Pro Val Ile
        195                 200                 205

Ala Tyr Ser Tyr Tyr Gly Asp Glu Gln Tyr Pro Arg Thr Ile Asn Ile
    210                 215                 220

Pro Tyr Pro Lys Ala Gly Ala Lys Asn Pro Val Val Arg Ile Phe Ile
225                 230                 235                 240

Ile Asp Thr Thr Tyr Pro Ala Tyr Val Gly Pro Gln Glu Val Pro Val
                245                 250                 255

Pro Ala Met Ile Ala Ser Ser Asp Tyr Tyr Phe Ser Trp Leu Thr Trp
            260                 265                 270

Val Thr Asp Glu Arg Val Cys Leu Gln Trp Leu Lys Arg Val Gln Asn
        275                 280                 285

Val Ser Val Leu Ser Ile Cys Asp Phe Arg Glu Asp Trp Gln Thr Trp
    290                 295                 300

Asp Cys Pro Lys Thr Gln Glu His Ile Glu Glu Ser Arg Thr Gly Trp
305                 310                 315                 320

Ala Gly Gly Phe Phe Val Ser Thr Pro Val Phe Ser Tyr Asp Ala Ile
                325                 330                 335

Ser Tyr Tyr Lys Ile Phe Ser Asp Lys Asp Gly Tyr Lys His Ile His
            340                 345                 350

Tyr Ile Lys Asp Thr Val Glu Asn Ala Ile Gln Ile Thr Ser Gly Lys
        355                 360                 365

Trp Glu Ala Ile Asn Ile Phe Arg Val Thr Gln Asp Ser Leu Phe Tyr
    370                 375                 380

Ser Ser Asn Glu Phe Glu Glu Tyr Pro Gly Arg Arg Asn Ile Tyr Arg
385                 390                 395                 400
```

```
Ile Ser Ile Gly Ser Tyr Pro Pro Ser Lys Lys Cys Val Thr Cys His
                405                 410                 415

Leu Arg Lys Glu Arg Cys Gln Tyr Tyr Thr Ala Ser Phe Ser Asp Tyr
            420                 425                 430

Ala Lys Tyr Tyr Ala Leu Val Cys Tyr Gly Pro Gly Ile Pro Ile Ser
        435                 440                 445

Thr Leu His Asp Gly Arg Thr Asp Gln Glu Ile Lys Ile Leu Glu Glu
    450                 455                 460

Asn Lys Glu Leu Glu Asn Ala Leu Lys Asn Ile Gln Leu Pro Lys Glu
465                 470                 475                 480

Glu Ile Lys Lys Leu Glu Val Asp Glu Ile Thr Leu Trp Tyr Lys Met
                485                 490                 495

Ile Leu Pro Pro Gln Phe Asp Arg Ser Lys Lys Tyr Pro Leu Leu Ile
            500                 505                 510

Gln Val Tyr Gly Gly Pro Cys Ser Gln Ser Val Arg Ser Val Phe Ala
        515                 520                 525

Val Asn Trp Ile Ser Tyr Leu Ala Ser Lys Glu Gly Met Val Ile Ala
    530                 535                 540

Leu Val Asp Gly Arg Gly Thr Ala Phe Gln Gly Asp Lys Leu Leu Tyr
545                 550                 555                 560

Ala Val Tyr Arg Lys Leu Gly Val Tyr Glu Val Glu Asp Gln Ile Thr
                565                 570                 575

Ala Val Arg Lys Phe Ile Glu Met Gly Phe Ile Asp Glu Lys Arg Ile
            580                 585                 590

Ala Ile Trp Gly Trp Ser Tyr Gly Gly Tyr Val Ser Ser Leu Ala Leu
        595                 600                 605

Ala Ser Gly Thr Gly Leu Phe Lys Cys Gly Ile Ala Val Ala Pro Val
    610                 615                 620

Ser Ser Trp Glu Tyr Tyr Ala Ser Val Tyr Thr Glu Arg Phe Met Gly
625                 630                 635                 640
```

Leu Pro Thr Lys Asp Asp Asn Leu Glu His Tyr Lys Asn Ser Thr Val
645 650 655

Met Ala Arg Ala Glu Tyr Phe Arg Asn Val Asp Tyr Leu Leu Ile His
660 665 670

Gly Thr Ala Asp Asp Asn Val His Phe Gln Asn Ser Ala Gln Ile Ala
675 680 685

Lys Ala Leu Val Asn Ala Gln Val Asp Phe Gln Ala Met Trp Tyr Ser
690 695 700

Asp Gln Asn His Gly Leu Ser Gly Leu Ser Thr Asn His Leu Tyr Thr
705 710 715 720

His Met Thr His Phe Leu Lys Gln Cys Phe Ser Leu Ser Asp
725 730

<210> 62
<211> 33
<212> PRT
<213> artificial sequence

<220>
<223> consensus sequence

<400> 62

Thr Asn Asn Pro Asn Ile Asn Tyr Ser Asn Tyr Asn Asp Glu Asn Asn
1 5 10 15

Gln Tyr Pro Asn Thr Asn Asn Asn Pro Tyr Pro Lys Ala Gly Ala Asn
20 25 30

Asn

<210> 63
<211> 68
<212> PRT
<213> artificial sequence

<220>
<223> consensus sequence

<400> 63

Val Asn Tyr Leu Leu Ile His Gly Thr Ala Asp Asp Asn Val His Phe
1 5 10 15

Gln Asn Ser Ala Gln Ile Asn Lys Ala Leu Val Asn Asn Asn Val Asp
20 25 30

Phe Gln Ala Met Trp Tyr Asn Asp Asn Asn His Gly Asn Asn Ser Asn
35 40 45

Asn Asn Asn Asn His Asn Tyr Thr His Met Asn His Phe Asn Lys Gln
50 55 60

Cys Phe Ser Leu
65

<210> 64
<211> 2310
<212> DNA
<213> Homo sapiens

<400> 64
atctggaaaa atgaagactt gggtaaaaat cgtatttgga gttgccacct ctgctgtgct 60

tgccttattg gtgatgtgca ttgtcttacg cccttcaaga gttcataact ctgaagaaaa 120

tacaatgaga gcactcacac tgaaggatat tttaaatgga acattttctt ataaaacatt 180

ttttccaaac tggatttcag gacaagaata tcttcatcaa tctgcagata acaatatagt 240

actttataat attgaaacag gacaatcata taccattttg agtaatagaa ccatgaaaag 300

tgtgaatgct tcaaattacg gcttatcacc tgatcggcaa tttgtatatc tagaaagtga 360

ttattcaaag ctttggagat actcttacac agcaacatat tacatctatg accttagcaa 420

tggagaattt gtaagaggaa atgagcttcc tcgtccaatt cagtatttat gctggtcgcc 480

tgttgggagt aaattagcat atgtctatca aaacaatatc tatttgaaac aaagaccagg 540

agatccacct tttcaaataa catttaatgg aagagaaaat aaaatattta atggaatccc 600

agactgggtt tatgaagagg aaatgcttgc tacaaaatat gctctctggt ggtctcctaa 660

tggaaaattt ttggcatatg cggaatttaa tgatacggat ataccagtta ttgcctattc 720

ctattatggc gatgaacaat atcctagaac aataaatatt ccatacccaa aggctggagc 780

taagaatccc gttgttcgga tatttattat cgataccact taccctgcgt atgtaggtcc 840

ccaggaagtg cctgttccag caatgatagc ctcaagtgat tattatttca gttggctcac 900

gtgggttact gatgaacgag tatgtttgca gtggctaaaa agagtccaga atgtttcggt 960

cctgtctata tgtgacttca gggaagactg gcagacatgg gattgtccaa agacccagga 1020

gcatatagaa gaaagcagaa ctggatgggc tggtggattc tttgtttcaa caccagtttt 1080

cagctatgat gccatttcgt actacaaaat atttagtgac aaggatggct acaaacatat 1140

tcactatatc aaagacactg tggaaaatgc tattcaaatt acaagtggca agtgggaggc 1200

cataaatata ttcagagtaa cacaggattc actgttttat tctagcaatg aatttgaaga 1260

ataccctgga agaagaaaca tctacagaat tagcattgga agctatcctc caagcaagaa 1320

gtgtgttact tgccatctaa ggaaagaaag gtgccaatat tacacagcaa gtttcagcga 1380

ctacgccaag tactatgcac ttgtctgcta cggcccaggc atccccattt ccacccttca 1440

tgatggacgc actgatcaag aaattaaaat cctggaagaa aacaaggaat tggaaaatgc 1500

tttgaaaaat atccagctgc ctaaagagga aattaagaaa cttgaagtag atgaaattac 1560

tttatggtac aagatgattc ttcctcctca atttgacaga tcaaagaagt atcccttgct 1620

aattcaagtg tatggtggtc cctgcagtca gagtgtaagg tctgtatttg ctgttaattg 1680

gatatcttat cttgcaagta aggaagggat ggtcattgcc ttggtggatg gtcgaggaac 1740

agctttccaa ggtgacaaac tcctctatgc agtgtatcga aagctgggtg tttatgaagt 1800

tgaagaccag attacagctg tcagaaaatt catagaaatg ggtttcattg atgaaaaaag 1860

aatagccata tggggctggt cctatggagg atacgtttca tcactggccc ttgcatctgg 1920

aactggtctt ttcaaatgtg gtatagcagt ggctccagtc tccagctggg aatattacgc 1980

gtctgtctac acagagagat tcatgggtct cccaacaaag gatgataatc ttgagcacta 2040

taagaattca actgtgatgg caagagcaga atatttcaga aatgtagact atcttctcat 2100

ccacggaaca gcagatgata atgtgcactt tcaaaactca gcacagattg ctaaagctct 2160

ggttaatgca caagtggatt tccaggcaat gtggtactct gaccagaacc acggcttatc 2220

cggcctgtcc acgaaccact tatacaccca catgacccac ttcctaaagc agtgtttctc 2280

tttgtcagac taaaaacgat gcagatgcaa 2310

<210> 65
<211> 58
<212> DNA
<213> artificial sequence

<220>
<223> PCR primer

<400> 65
ggtgacgcgg cccagccggc cacaaagaga gctcttaccc tgaaggatat tttaaatg 58

<210> 66
<211> 766
<212> PRT
<213> Homo sapiens

<400> 66

Met Lys Thr Pro Trp Lys Val Leu Leu Gly Leu Leu Gly Ala Ala Ala
1 5 10 15

Leu Val Thr Ile Ile Thr Val Pro Val Val Leu Leu Asn Lys Gly Thr
20 25 30

Asp Asp Ala Thr Ala Asp Ser Arg Lys Thr Tyr Thr Leu Thr Asp Tyr
35 40 45

Leu Lys Asn Thr Tyr Arg Leu Lys Leu Tyr Ser Leu Arg Trp Ile Ser
50 55 60

Asp His Glu Tyr Leu Tyr Lys Gln Glu Asn Asn Ile Leu Val Phe Asn
65 70 75 80

Ala Glu Tyr Gly Asn Ser Ser Val Phe Leu Glu Asn Ser Thr Phe Asp
85 90 95

Glu Phe Gly His Ser Ile Asn Asp Tyr Ser Ile Ser Pro Asp Gly Gln
100 105 110

Phe Ile Leu Leu Glu Tyr Asn Tyr Val Lys Gln Trp Arg His Ser Tyr
115 120 125

Thr Ala Ser Tyr Asp Ile Tyr Asp Leu Asn Lys Arg Gln Leu Ile Thr
130 135 140

Glu Glu Arg Ile Pro Asn Asn Thr Gln Trp Val Thr Trp Ser Pro Val
145 150 155 160

Gly His Lys Leu Ala Tyr Val Trp Asn Asn Asp Ile Tyr Val Lys Ile
165 170 175

Glu Pro Asn Leu Pro Ser Tyr Arg Ile Thr Trp Thr Gly Lys Glu Asp
180 185 190

Ile Ile Tyr Asn Gly Ile Thr Asp Trp Val Tyr Glu Glu Glu Val Phe
195 200 205

Ser Ala Tyr Ser Ala Leu Trp Trp Ser Pro Asn Gly Thr Phe Leu Ala
210 215 220

Tyr Ala Gln Phe Asn Asp Thr Glu Val Pro Leu Ile Glu Tyr Ser Phe
225 230 235 240

Tyr Ser Asp Glu Ser Leu Gln Tyr Pro Lys Thr Val Arg Val Pro Tyr
245 250 255

Pro Lys Ala Gly Ala Val Asn Pro Thr Val Lys Phe Phe Val Val Asn
260 265 270

Thr Asp Ser Leu Ser Ser Val Thr Asn Ala Thr Ser Ile Gln Ile Thr
275 280 285

Ala Pro Ala Ser Met Leu Ile Gly Asp His Tyr Leu Cys Asp Val Thr
290 295 300

Trp Ala Thr Gln Glu Arg Ile Ser Leu Gln Trp Leu Arg Arg Ile Gln
305 310 315 320

Asn Tyr Ser Val Met Asp Ile Cys Asp Tyr Asp Glu Ser Ser Gly Arg
325 330 335

Trp Asn Cys Leu Val Ala Arg Gln His Ile Glu Met Ser Thr Thr Gly
340 345 350

Trp Val Gly Arg Phe Arg Pro Ser Glu Pro His Phe Thr Leu Asp Gly
355 360 365

Asn Ser Phe Tyr Lys Ile Ile Ser Asn Glu Glu Gly Tyr Arg His Ile
370 375 380

Cys Tyr Phe Gln Ile Asp Lys Lys Asp Cys Thr Phe Ile Thr Lys Gly
385 390 395 400

Thr Trp Glu Val Ile Gly Ile Glu Ala Leu Thr Ser Asp Tyr Leu Tyr
405 410 415

Tyr Ile Ser Asn Glu Tyr Lys Gly Met Pro Gly Gly Arg Asn Leu Tyr
420 425 430

Lys Ile Gln Leu Ser Asp Tyr Thr Lys Val Thr Cys Leu Ser Cys Glu
435 440 445

Leu Asn Pro Glu Arg Cys Gln Tyr Tyr Ser Val Ser Phe Ser Lys Glu
450 455 460

Ala Lys Tyr Tyr Gln Leu Arg Cys Ser Gly Pro Gly Leu Pro Leu Tyr
465 470 475 480

Thr Leu His Ser Ser Val Asn Asp Lys Gly Leu Arg Val Leu Glu Asp
485 490 495

Asn Ser Ala Leu Asp Lys Met Leu Gln Asn Val Gln Met Pro Ser Lys
500 505 510

Lys Leu Asp Phe Ile Ile Leu Asn Glu Thr Lys Phe Trp Tyr Gln Met
515 520 525

Ile Leu Pro Pro His Phe Asp Lys Ser Lys Lys Tyr Pro Leu Leu Leu
530 535 540

Asp Val Tyr Ala Gly Pro Cys Ser Gln Lys Ala Asp Thr Val Phe Arg
545 550 555 560

Leu Asn Trp Ala Thr Tyr Leu Ala Ser Thr Glu Asn Ile Ile Val Ala
565 570 575

Ser Phe Asp Gly Arg Gly Ser Gly Tyr Gln Gly Asp Lys Ile Met His
580 585 590

Ala Ile Asn Arg Arg Leu Gly Thr Phe Glu Val Glu Asp Gln Ile Glu
595 600 605

Ala Ala Arg Gln Phe Ser Lys Met Gly Phe Val Asp Asn Lys Arg Ile
610 615 620

Ala Ile Trp Gly Trp Ser Tyr Gly Gly Tyr Val Thr Ser Met Val Leu
625 630 635 640

Gly Ser Gly Ser Gly Val Phe Lys Cys Gly Ile Ala Val Ala Pro Val
645 650 655

Ser Arg Trp Glu Tyr Tyr Asp Ser Val Tyr Thr Glu Arg Tyr Met Gly
660 665 670

Leu Pro Thr Pro Glu Asp Asn Leu Asp His Tyr Arg Asn Ser Thr Val
675 680 685

Met Ser Arg Ala Glu Asn Phe Lys Gln Val Glu Tyr Leu Leu Ile His
690 695 700

Gly Thr Ala Asp Asp Asn Val His Phe Gln Gln Ser Ala Gln Ile Ser
705 710 715 720

Lys Ala Leu Val Asp Val Gly Val Asp Phe Gln Ala Met Trp Tyr Thr
725 730 735

Asp Glu Asp His Gly Ile Ala Ser Ser Thr Ala His Gln His Ile Tyr
740 745 750

Thr His Met Ser His Phe Ile Lys Gln Cys Phe Ser Leu Pro
755 760 765

<210> 67
<211> 3913
<212> DNA
<213> Homo sapiens

<400> 67
ctttcactgg caagagacgg agtcctgggt ttcagttcca gttgcctgcg gtgggctgtg 60

tgagtttgcc aaagtcccct gccctctctg ggtctcggtt ccctcgcctg tccacgtgag 120

gttggaggag ctgaacgccg acgtcatttt tagctaagag ggagcagggt ccccgagtcg 180

ccggcccagg gtctgcgcat ccgaggccgc gcgccctttc ccctccccca cggctcctcc 240

gggccccgca ctctgcgccc cggctgccgc ccagcgccct acaccgccct caggggccc 300

tcgcgggctc cccccggccg ggatgccagt gccccgcgcc acgcgcgcct gctcccgcgc 360

cgcctgccct gcagcctgcc cgcggcgcct ttatacccag cgggctcggc gctcactaat 420

gtttaactcg gggccgaaac ttgccagcgg cgagtgactc caccgcccgg agcagcggtg 480

caggacgcgc gtctccgccg cccgcggtga cttctgcctg cgctccttct ctgaacgctc 540

acttccgagg agacgccgac gatgaagaca ccgtggaagg ttcttctggg actgctgggt 600

gctgctgcgc ttgtcaccat catcaccgtg cccgtggttc tgctgaacaa aggcacagat 660

gatgctacag ctgacagtcg caaaacttac actctaactg attacttaaa aaatacttat 720

agactgaagt tatactcctt aagatggatt tcagatcatg aatatctcta caaacaagaa 780

aataatatct tggtattcaa tgctgaatat ggaaacagct cagttttctt ggagaacagt 840

acatttgatg agtttggaca ttctatcaat gattattcaa tatctcctga tgggcagttt 900

attctcttag aatacaacta cgtgaagcaa tggaggcatt cctacacagc ttcatatgac 960

atttatgatt taaataaaag gcagctgatt acagaagaga ggattccaaa caacacacag 1020

tgggtcacat ggtcaccagt gggtcataaa ttggcatatg tttggaacaa tgacatttat 1080

gttaaaattg aaccaaattt accaagttac agaatcacat ggacggggaa agaagatata 1140

atatataatg gaataactga ctgggtttat gaagaggaag tcttcagtgc ctactctgct 1200

ctgtggtggt ctccaaacgg cacttttta gcatatgccc aatttaacga cacagaagtc 1260

ccacttattg aatactcctt ctactctgat gagtcactgc agtacccaaa gactgtacgg 1320

gttccatatc caaaggcagg agctgtgaat ccaactgtaa agttctttgt tgtaaataca 1380

gactctctca gctcagtcac caatgcaact tccatacaaa tcactgctcc tgcttctatg 1440

ttgatagggg atcactactt gtgtgatgtg acatgggcaa cacaagaaag aatttctttg 1500

cagtggctca ggaggattca gaactattcg gtcatggata tttgtgacta tgatgaatcc 1560

agtggaagat ggaactgctt agtggcacgg caacacattg aaatgagtac tactggctgg 1620

gttggaagat ttaggccttc agaacctcat tttacccttg atggtaatag cttctacaag 1680

atcatcagca atgaagaagg ttacagacac atttgctatt tccaaataga taaaaaagac 1740

tgcacattta ttacaaaagg cacctgggaa gtcatcggga tagaagctct aaccagtgat 1800

tatctatact acattagtaa tgaatataaa ggaatgccag gaggaaggaa tctttataaa 1860

atccaactta gtgactatac aaaagtgaca tgcctcagtt gtgagctgaa tccggaaagg 1920

tgtcagtact attctgtgtc attcagtaaa gaggcgaagt attatcagct gagatgttcc 1980

ggtcctggtc tgcccctcta tactctacac agcagcgtga atgataaagg gctgagagtc 2040

ctggaagaca attcagcttt ggataaaatg ctgcagaatg tccagatgcc ctccaaaaaa 2100

ctggacttca ttattttgaa tgaaacaaaa ttttggtatc agatgatctt gcctcctcat 2160

tttgataaat ccaagaaata tcctctacta ttagatgtgt atgcaggccc atgtagtcaa 2220

aaagcagaca ctgtcttcag actgaactgg gccacttacc ttgcaagcac agaaaacatt 2280

atagtagcta gctttgatgg cagaggaagt ggttaccaag gagataagat catgcatgca 2340

atcaacagaa gactgggaac atttgaagtt gaagatcaaa ttgaagcagc cagacaattt 2400

tcaaaaatgg gatttgtgga caacaaacga attgcaattt ggggctggtc atatggaggg 2460

tacgtaacct caatggtcct gggatcggga agtggcgtgt tcaagtgtgg aatagccgtg 2520

gcgcctgtat cccggtggga gtactatgac tcagtgtaca cagaacgtta catgggtctc 2580

ccaactccag aagacaacct tgaccattac agaaattcaa cagtcatgag cagagctgaa 2640

aattttaaac aagttgagta cctccttatt catggaacag cagatgataa cgttcacttt 2700

cagcagtcag ctcagatctc caaagccctg gtcgatgttg gagtggattt ccaggcaatg 2760

tggtatactg atgaagacca tggaatagct agcagcacag cacaccaaca tatatatacc 2820

cacatgagcc acttcataaa acaatgtttc tctttacctt agcacctcaa aataccatgc 2880

catttaaagc ttattaaaac tcatttttgt tttcattatc tcaaaactgc actgtcaaga 2940

tgatgatgat ctttaaaata cacactcaaa tcaagaaact taaggttacc tttgttccca 3000

aatttcatac ctatcatctt aagtagggac ttctgtcttc acaacagatt attaccttac 3060

agaagtttga attatccggt cgggttttat tgtttaaaat catttctgca tcagctgctg 3120

aaacaacaaa taggaattgt ttttatggag gctttgcata gattccctga gcaggatttt 3180

aatctttttc taactggact ggttcaaatg ttgttctctt ctttaaaggg atggcaagat 3240

gtgggcagtg atgtcactag ggcagggaca ggataagagg gattagggag agaagatagc 3300

agggcatggc tgggaaccca agtccaagca taccaacacg agcaggctac tgtcagctcc 3360

cctcggagaa gagctgttca cagccagact ggcacagttt tctgagaaag actattcaaa 3420

cagtctcagg aaatcaaata tgcaaagcac tgacttctaa gtaaaaccac agcagttgaa 3480

aagactccaa agaaatgtaa gggaaactgc cagcaacgca ggcccccagg tgccagttat 3540

ggctataggt gctacaaaaa cacagcaagg gtgatgggaa agcattgtaa atgtgctttt 3600

aaaaaaaaat actgatgttc ctagtgaaag aggcagcttg aaactgagat gtgaacacat 3660

cagcttgccc tgttaaaaga tgaaaatatt tgtatcacaa atcttaactt gaaggagtcc 3720

ttgcatcaat ttttcttatt tcatttcttt gagtgtctta attaaaagaa tattttaact 3780

tccttggact cattttaaaa aatggaacat aaaatacaat gttatgtatt attattccca 3840

ttctacatac tatggaattt ctcccagtca tttaataaat gtgccttcat tttttcagaa 3900

aaaaaaaaaa aaa 3913

<210> 68
<211> 3482
<212> DNA
<213> Mus musculus

<400> 68
ccacgcgtcc ggtctctttt agcaaagggg gttcgctgtt ccccttatcc ctcactcatg 60

ggggccctgc gtgctacttc ctggctcgcc tagtggtcct accgcgctgg gtcggctgcc 120

ccgcgctcac actgtgcacc gaagcccgcc ttgcgctaac taatgtttaa ctcaggccga 180

aacttggcgg cgagctcagg gtgactgcgt gcagagcagc ggcgcaggac gtctgtctct 240

gcgcgcagtg acttctgcct gcgctcaagc tacagagttc agtttcaagg agccgcccga 300

ccatgaagac accgtggaag gttcttctgg gactgcttgg tgtcgctgcg cttgtcacca 360

tcatcaccgt gccaatagtt ctgctgagca aagatgaagc ggcagctgac agccgcagaa 420

cgtattcact agctgactat ttaaagagta cctttcgggt caagtcctac tctttgtggt 480

gggtttcaga ctttgaatac ctctacaaac aagagaacaa tatcttgctg ctcaatgctg 540

aacatggaaa cagctccatt ttcttggaga acagtacctt tgaaagcttt ggatatcatt 600

cagtgtcacc tgaccgactg tttgttctct tggaatacaa ctacgtgaag caatggagac 660

attcctacac agcttcatac aacatttatg atgtgaataa aagacagctg atcacagaag 720

agaagattcc aaataataca cagtggatca catggtcacc agaaggtcat aagttggcat 780

atgtctggaa gaatgatatt tacgttaaag ttgaaccaca cttacctagt cataggatca 840

catcgacagg agaagaaaat gtaatatata atggaataac tgactgggtt tatgaagagg 900

aagtcttcgg tgcctactct gcactgtggt ggtctccaaa caacacgttt ctagcttatg 960

cccagtttaa cgacacagga gtgccgctca ttgaatactc cttctattct gatgagtcac 1020

tgcagtaccc caagacagtg tggattccat acccaaaggc aggagctgtg aatccaactg 1080

taaagttctt tattgtaaat atagactctc tcagctcatc ctctagtgcg gctcccatcc 1140

aaatccctgc tcctgcatct gtggcaagag gggatcacta tttatgtgat gtggtgtggg 1200

ctacagaaga aagaatttca ctacagtggc tcaggaggat tcagaactat tccgtgatgg 1260

ctatctgtga ctatgataag atcaacctaa cgtggaactg tccatccgag cagcagcacg 1320

ttgaaatgag taccacgggc tgggtcggaa gatttaggcc cgcagaacct cacttcacct 1380

ctgatggaag cagcttctat aagatcatca gcgacaaaga tggctataaa cacatctgcc 1440

acttcccgaa agataagaaa gactgtacat ttattacaaa aggagcctgg gaagtcatta 1500

gtatcgaagc tctgaccagc gattatctat actacattag taaccaatat aaagaaatgc 1560

caggaggaag aaatctctat aaaattcaac ttactgacca cacaaatgtg aagtgcctta 1620

gttgtgacct gaatccagaa agatgtcagt attatgcagt atcatttagt aaagaggcaa 1680

agtactatca gctgggatgt tggggccccg gtctgcccct ctacactcta catcgtagca 1740

cggatcataa agagctgcga gtcctggaag acaattctgc tttggataga atgctgcagg 1800

atgtccagat gccttcaaaa aaattggact tcattgtttt gaatgaaaca agattttggt 1860

atcaaatgat cttgcccccc cattttgata aatccaagaa atatcctcta ctattagatg 1920

tatatgcagg tccctgtagt caaaaagcag atgcttcctt cagactgaac tgggccactt 1980

accttgcaag tacagaaaac atcatagtag ctagctttga cggcagagga agtggttacc 2040

aaggagataa gatcatgcat gcaatcaaca gaagattggg aacactggaa gttgaagatc 2100

aaattgaagc agcgaggcaa tttgtaaaaa tgggatttgt ggacagcaag cgagttgcaa 2160

tttggggctg gtcatatgga gggtatgtaa cctcaatggt cctgggatcg ggaagtggcg 2220

tgttcaagtg cggaatagct gtggcacctg tgtcacggtg ggagtactat gactcagtgt 2280

acacagagcg ttacatgggt ctcccaattc cagaagacaa ccttgaccat tacaggaact 2340

caacagtcat gagcagagct gaacatttta aacaagttga gtacctcctt attcatggaa 2400

cggcagatga taatgttcac tttcagcagt cagctcagat ctccaaagcc ctggtggatg 2460

ctggcgtgga tttccaagca atgtggtaca cggatgaaga ccacgggatc gctagcagca 2520

cagctcacca gcacatctat tcccacatga gccatttcct ccagcagtgc ttctccttac 2580

actagcacag catagctctc catagcttat ttaagaccac atttgttctc attatctcaa 2640

aagtgcactg ttaagatgaa gatcttaata atgttgcatt gagacatttc aggctgcttt 2700

ctccagtttt acacctgcaa tcctaactaa ggatgcctgt ccccagaaca gattactacc 2760

ttagagcaat ttggattttc ccctctgttt tgtttatcat ttaaaatcat ttccacatca 2820

gctgctgaaa caacaaatat aaattatttt tgcaagagct atgcatagat ttcctgagca 2880

gaatttcaat ttttttttt tccttactag actggtccaa atctcgtttc ccttatttaa 2940

gtgggtgaca agatgtgggt aatgatgtca ttagggcagc aacaagagaa gagggagcag 3000

ggagtatggc tagaaaccca ggtccaagca tacaaaccaa ccaggctact gtcagctcgc 3060

ctcgagaaga gctgttcact gccagactgg cacagttttc tgagaaagac tattcaaaca 3120

gtctcaggaa atcatatatg caaagcactg acttctaagt aaaaccacag cagttgaata 3180

gactccaaag aaatgcaagg gacgctgcca gcaatgtaag ggccccaggt gccagttatg 3240

gctataggtg ctacaaaaac acagcaaggg tgatgggaaa gcattgtaaa tgtgcttttg 3300

aaaattaccg atgtctccta gtgaaaagag gcagcttgga acatatcgac ttgccctgtt 3360

aaaagttgaa aatatttgtg tcacaaattc taacatgaaa gaatacttgc attagttttt 3420

cctacttcat ttctttgagc attttaatta aaatatttta acttcaaaaa aaaaaaaaaa 3480

ag 3482

<210> 69
<211> 760
<212> PRT
<213> Mus musculus

<400> 69

Met Lys Thr Pro Trp Lys Val Leu Leu Gly Leu Leu Gly Val Ala Ala
1 5 10 15

Leu Val Thr Ile Ile Thr Val Pro Ile Val Leu Leu Ser Lys Asp Glu
20 25 30

Ala Ala Ala Asp Ser Arg Arg Thr Tyr Ser Leu Ala Asp Tyr Leu Lys
35 40 45

Ser Thr Phe Arg Val Lys Ser Tyr Ser Leu Trp Trp Val Ser Asp Phe
50 55 60

Glu Tyr Leu Tyr Lys Gln Glu Asn Asn Ile Leu Leu Leu Asn Ala Glu
65 70 75 80

His Gly Asn Ser Ser Ile Phe Leu Glu Asn Ser Thr Phe Glu Ser Phe
85 90 95

Gly Tyr His Ser Val Ser Pro Asp Arg Leu Phe Val Leu Leu Glu Tyr
100 105 110

Asn Tyr Val Lys Gln Trp Arg His Ser Tyr Thr Ala Ser Tyr Asn Ile
115 120 125

Tyr Asp Val Asn Lys Arg Gln Leu Ile Thr Glu Glu Lys Ile Pro Asn
130 135 140

Asn Thr Gln Trp Ile Thr Trp Ser Pro Glu Gly His Lys Leu Ala Tyr
145 150 155 160

Val Trp Lys Asn Asp Ile Tyr Val Lys Val Glu Pro His Leu Pro Ser
165 170 175

His Arg Ile Thr Ser Thr Gly Glu Glu Asn Val Ile Tyr Asn Gly Ile
180 185 190

Thr Asp Trp Val Tyr Glu Glu Glu Val Phe Gly Ala Tyr Ser Ala Leu
195 200 205

Trp Trp Ser Pro Asn Asn Thr Phe Leu Ala Tyr Ala Gln Phe Asn Asp
210 215 220

Thr Gly Val Pro Leu Ile Glu Tyr Ser Phe Tyr Ser Asp Glu Ser Leu
225 230 235 240

Gln Tyr Pro Lys Thr Val Trp Ile Pro Tyr Pro Lys Ala Gly Ala Val
245 250 255

Asn Pro Thr Val Lys Phe Phe Ile Val Asn Ile Asp Ser Leu Ser Ser
260 265 270

Ser Ser Ser Ala Ala Pro Ile Gln Ile Pro Ala Pro Ala Ser Val Ala
275 280 285

Arg Gly Asp His Tyr Leu Cys Asp Val Val Trp Ala Thr Glu Glu Arg
290 295 300

Ile Ser Leu Gln Trp Leu Arg Arg Ile Gln Asn Tyr Ser Val Met Ala
305 310 315 320

Ile Cys Asp Tyr Asp Lys Ile Asn Leu Thr Trp Asn Cys Pro Ser Glu
325 330 335

Gln Gln His Val Glu Met Ser Thr Thr Gly Trp Val Gly Arg Phe Arg
340 345 350

Pro Ala Glu Pro His Phe Thr Ser Asp Gly Ser Ser Phe Tyr Lys Ile
355 360 365

Ile Ser Asp Lys Asp Gly Tyr Lys His Ile Cys His Phe Pro Lys Asp
370 375 380

Lys Lys Asp Cys Thr Phe Ile Thr Lys Gly Ala Trp Glu Val Ile Ser
385 390 395 400

Ile Glu Ala Leu Thr Ser Asp Tyr Leu Tyr Tyr Ile Ser Asn Gln Tyr

405 410 415

Lys Glu Met Pro Gly Gly Arg Asn Leu Tyr Lys Ile Gln Leu Thr Asp
420 425 430

His Thr Asn Val Lys Cys Leu Ser Cys Asp Leu Asn Pro Glu Arg Cys
435 440 445

Gln Tyr Tyr Ala Val Ser Phe Ser Lys Glu Ala Lys Tyr Tyr Gln Leu
450 455 460

Gly Cys Trp Gly Pro Gly Leu Pro Leu Tyr Thr Leu His Arg Ser Thr
465 470 475 480

Asp His Lys Glu Leu Arg Val Leu Glu Asp Asn Ser Ala Leu Asp Arg
485 490 495

Met Leu Gln Asp Val Gln Met Pro Ser Lys Lys Leu Asp Phe Ile Val
500 505 510

Leu Asn Glu Thr Arg Phe Trp Tyr Gln Met Ile Leu Pro Pro His Phe
515 520 525

Asp Lys Ser Lys Lys Tyr Pro Leu Leu Leu Asp Val Tyr Ala Gly Pro
530 535 540

Cys Ser Gln Lys Ala Asp Ala Ser Phe Arg Leu Asn Trp Ala Thr Tyr
545 550 555 560

Leu Ala Ser Thr Glu Asn Ile Ile Val Ala Ser Phe Asp Gly Arg Gly
565 570 575

Ser Gly Tyr Gln Gly Asp Lys Ile Met His Ala Ile Asn Arg Arg Leu
580 585 590

Gly Thr Leu Glu Val Glu Asp Gln Ile Glu Ala Ala Arg Gln Phe Val
595 600 605

Lys Met Gly Phe Val Asp Ser Lys Arg Val Ala Ile Trp Gly Trp Ser
610 615 620

Tyr Gly Gly Tyr Val Thr Ser Met Val Leu Gly Ser Gly Ser Gly Val
625 630 635 640

Phe Lys Cys Gly Ile Ala Val Ala Pro Val Ser Arg Trp Glu Tyr Tyr
645 650 655

Asp Ser Val Tyr Thr Glu Arg Tyr Met Gly Leu Pro Ile Pro Glu Asp
660 665 670

Asn Leu Asp His Tyr Arg Asn Ser Thr Val Met Ser Arg Ala Glu His
675 680 685

Phe Lys Gln Val Glu Tyr Leu Leu Ile His Gly Thr Ala Asp Asp Asn
690 695 700

Val His Phe Gln Gln Ser Ala Gln Ile Ser Lys Ala Leu Val Asp Ala
705 710 715 720

Gly Val Asp Phe Gln Ala Met Trp Tyr Thr Asp Glu Asp His Gly Ile
725 730 735

Ala Ser Ser Thr Ala His Gln His Ile Tyr Ser His Met Ser His Phe
740 745 750

Leu Gln Gln Cys Phe Ser Leu His
755 760

<210> 70
<211> 723
<212> PRT
<213> Homo sapiens

<400> 70

Thr Met Arg Ala Leu Thr Leu Lys Asp Ile Leu Asn Gly Thr Phe Ser
1 5 10 15

Tyr Lys Thr Phe Phe Pro Asn Trp Ile Ser Gly Gln Glu Tyr Leu His
20 25 30

Gln Ser Ala Asp Asn Asn Ile Val Leu Tyr Asn Ile Glu Thr Gly Gln
35 40 45

Ser Tyr Thr Ile Leu Ser Asn Arg Thr Met Lys Ser Val Asn Ala Ser
50 55 60

Asn Tyr Gly Leu Ser Pro Asp Arg Gln Phe Val Tyr Leu Glu Ser Asp
65 70 75 80

Tyr Ser Lys Leu Trp Arg Tyr Ser Tyr Thr Ala Thr Tyr Tyr Ile Tyr
85 90 95

Asp Leu Ser Asn Gly Glu Phe Val Arg Gly Asn Glu Leu Pro Arg Pro
100 105 110

Ile Gln Tyr Leu Cys Trp Ser Pro Val Gly Ser Lys Leu Ala Tyr Val
115 120 125

Tyr Gln Asn Asn Ile Tyr Leu Lys Gln Arg Pro Gly Asp Pro Pro Phe
130 135 140

Gln Ile Thr Phe Asn Gly Arg Glu Asn Lys Ile Phe Asn Gly Ile Pro
145 150 155 160

Asp Trp Val Tyr Glu Glu Glu Met Leu Ala Thr Lys Tyr Ala Leu Trp
165 170 175

Trp Ser Pro Asn Gly Lys Phe Leu Ala Tyr Ala Glu Phe Asn Asp Thr
180 185 190

Asp Ile Pro Val Ile Ala Tyr Ser Tyr Tyr Gly Asp Glu Gln Tyr Pro
195 200 205

Arg Thr Ile Asn Ile Pro Tyr Pro Lys Ala Gly Ala Lys Asn Pro Val
210 215 220

Val Arg Ile Phe Ile Ile Asp Thr Thr Tyr Pro Ala Tyr Val Gly Pro
225 230 235 240

Gln Glu Val Pro Val Pro Ala Met Ile Ala Ser Ser Asp Tyr Tyr Phe
245 250 255

Ser Trp Leu Thr Trp Val Thr Asp Glu Arg Val Cys Leu Gln Trp Leu
260 265 270

Lys Arg Val Gln Asn Val Ser Val Leu Ser Ile Cys Asp Phe Arg Glu
275 280 285

Asp Trp Gln Thr Trp Asp Cys Pro Lys Thr Gln Glu His Ile Glu Glu
290 295 300

Ser Arg Thr Gly Trp Ala Gly Gly Phe Phe Val Ser Thr Pro Val Phe
305 310 315 320

Ser Tyr Asp Ala Ile Ser Tyr Tyr Lys Ile Phe Ser Asp Lys Asp Gly
325 330 335

Tyr Lys His Ile His Tyr Ile Lys Asp Thr Val Glu Asn Ala Ile Gln
340 345 350

```
Ile Thr Ser Gly Lys Trp Glu Ala Ile Asn Ile Phe Arg Val Thr Gln
        355                 360                 365

Asp Ser Leu Phe Tyr Ser Ser Asn Glu Phe Glu Glu Tyr Pro Gly Arg
    370                 375                 380

Arg Asn Ile Tyr Arg Ile Ser Ile Gly Ser Tyr Pro Pro Ser Lys Lys
385                 390                 395                 400

Cys Val Thr Cys His Leu Arg Lys Glu Arg Cys Gln Tyr Tyr Thr Ala
                405                 410                 415

Ser Phe Ser Asp Tyr Ala Lys Tyr Tyr Ala Leu Val Cys Tyr Gly Pro
            420                 425                 430

Gly Ile Pro Ile Ser Thr Leu His Asp Gly Arg Thr Asp Gln Glu Ile
        435                 440                 445

Lys Ile Leu Glu Glu Asn Lys Glu Leu Glu Asn Ala Leu Lys Asn Ile
    450                 455                 460

Gln Leu Pro Lys Glu Glu Ile Lys Lys Leu Glu Val Asp Glu Ile Thr
465                 470                 475                 480

Leu Trp Tyr Lys Met Ile Leu Pro Pro Gln Phe Asp Arg Ser Lys Lys
                485                 490                 495

Tyr Pro Leu Leu Ile Gln Val Tyr Gly Gly Pro Cys Ser Gln Ser Val
            500                 505                 510

Arg Ser Val Phe Ala Val Asn Trp Ile Ser Tyr Leu Ala Ser Lys Glu
        515                 520                 525

Gly Met Val Ile Ala Leu Val Asp Gly Arg Gly Thr Ala Phe Gln Gly
    530                 535                 540

Asp Lys Leu Leu Tyr Ala Val Tyr Arg Lys Leu Gly Val Tyr Glu Val
545                 550                 555                 560

Glu Asp Gln Ile Thr Ala Val Arg Lys Phe Ile Glu Met Gly Phe Ile
                565                 570                 575

Asp Glu Lys Arg Ile Ala Ile Trp Gly Trp Ser Tyr Gly Gly Tyr Val
            580                 585                 590

Ser Ser Leu Ala Leu Ala Ser Gly Thr Gly Leu Phe Lys Cys Gly Ile
        595                 600                 605
```

Ala Val Ala Pro Val Ser Ser Trp Glu Tyr Tyr Ala Ser Val Tyr Thr
610 615 620

Glu Arg Phe Met Gly Leu Pro Thr Lys Asp Asp Asn Leu Glu His Tyr
625 630 635 640

Lys Asn Ser Thr Val Met Ala Arg Ala Glu Tyr Phe Arg Asn Val Asp
645 650 655

Tyr Leu Leu Ile His Gly Thr Ala Asp Asp Asn Val His Phe Gln Asn
660 665 670

Ser Ala Gln Ile Ala Lys Ala Leu Val Asn Ala Gln Val Asp Phe Gln
675 680 685

Ala Met Trp Tyr Ser Asp Gln Asn His Gly Leu Ser Gly Leu Ser Thr
690 695 700

Asn His Leu Tyr Thr His Met Thr His Phe Leu Lys Gln Cys Phe Ser
705 710 715 720

Leu Ser Asp

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US2005/000709

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N9/48 C07K14/705 A61K38/48 A61P37/06 A61P37/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE, CHEM ABS Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99/47152 A (SLOAN KETTERING INSTITUTE FOR CANCER RESEARCH; HOUGHTON, ALAN, N; WESL) 23 September 1999 (1999-09-23) page 3, line 11 - page 4, line 5; claims 26-30 page 20, line 25 - page 22, line 29	77,82,84
X	WO 97/34927 A (LUDWIG INSTITUTE FOR CANCER RESEARCH; BOEHRINGER INGELHEIM INTERNATION) 25 September 1997 (1997-09-25) claims page 1, line 9 - line 31	134
X	US 2002/034789 A1 (ZIMMERMANN RAINER ET AL) 21 March 2002 (2002-03-21) claims paragraph '0002!	134

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 May 2005	09/06/2005

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Böhmerova, E

Form PCT/ISA/210 (second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US2005/000709

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ROSENBLUM JONATHAN S ET AL: "Prolyl peptidases: A serine protease subfamily with high potential for drug discovery." CURRENT OPINION IN CHEMICAL BIOLOGY, vol. 7, no. 4, August 2003 (2003-08), pages 496-504, XP002329208 ISSN: 1367-5931 page 499, right-hand column, paragraph 2 - page 500, left-hand column, paragraph 1	1-76

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US2005/000709

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9947152 A	23-09-1999	AU 3357299 A	11-10-1999
		WO 9947152 A2	23-09-1999
WO 9734927 A	25-09-1997	US 5767242 A	16-06-1998
		AU 729369 B2	01-02-2001
		AU 2330297 A	10-10-1997
		BG 102781 A	30-09-1999
		BR 9708100 A	04-01-2000
		CA 2242342 A1	25-09-1997
		CN 1214052 A	14-04-1999
		CZ 9802996 A3	13-01-1999
		EE 9800306 A	15-02-1999
		EP 0960127 A1	01-12-1999
		HU 0104468 A2	29-04-2002
		JP 2000507100 T	13-06-2000
		NO 984298 A	17-09-1998
		NZ 331758 A	28-01-2000
		NZ 335543 A	30-03-2001
		PL 328947 A1	01-03-1999
		SK 126798 A3	07-05-1999
		TR 9801845 T2	21-12-1998
		WO 9734927 A1	25-09-1997
		US 5965373 A	12-10-1999
US 2002034789 A1	21-03-2002	US 5965373 A	12-10-1999
		US 5587299 A	24-12-1996
		AT 253113 T	15-11-2003
		AU 685273 B2	15-01-1998
		AU 2359495 A	16-11-1995
		CA 2188264 A1	02-11-1995
		DE 69532033 D1	04-12-2003
		DE 69532033 T2	08-07-2004
		EP 0763105 A1	19-03-1997
		JP 10504443 T	06-05-1998
		WO 9529233 A1	02-11-1995
		US 5767242 A	16-06-1998